The University of HULL

# Studies on The Factors That Improve The Outcome of IVF-ET

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*To my father, my husband Ali Abdullah Khalid and my daughters Zainab, Yasmin and Mayada for their support and confidence in me*

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## Notes on the text:

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Journal of Clinical Endocrinology and Metabolism. Schlegel and Girardi (1997). In Vitro Fertilization for Male Factor Infertility. Figure (1.5), (1.6) ©http://jcem.endojournals.org/cgi/content/full/82/3/709 Accessed on 12/07/06, at 3:42pm.

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Mencken, J., Trussel, J., and Larsen, U. (1986). Science: **233:** pp. 1389-1394 .figure (2.21). [Copyright (1986) APAS.]

Templeton, A. Morris, K.J., and Parslow W. (1996).Lancet. Figure (2.22).

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Andres Salumets (2003)Department of Biosciences, Division of Animal Physiology Faculty of Science, University of Helsinki, Finland and Infertility Clinic, The Family Federation of Finland Helsinki, Finland .Figure (2.24).

Toya, M., Saito, H., Ohta, N., Saito, T.,Kaneko, T., and Hiroi, M. (2000); .Fertil. Steril. Figure (2.25).

Kuivasaari, P., Hippelainen, M., Anttilla, M., and Heinonen, S. (2005). Hum.Reprod. figure (2.26).

Reproductive endocrinology and infertility 866.HRC.4ivf-(472-4483) live births per transfer for fresh embryos from own and donor eggs, by age of recipient, 1995. http://www.havingbabies.com/age-infertility-ovarian.html (Accessed on 19/8/2006 at 5.34pm). Ovarian Aging and Infertility Figure (3.5).

Kojima, K., Nomiyama M·, Kumamoto T, Matsumoto Y., and Iwasaka T.,¡ (2001).Transvaginal ultrasound-guided embryo transfer improves pregnancy and implantation rates after IVF. Hum. Reprod. Vol. **16**, No. **12**:pp.2578-2582.(modified figure) Figure (4.1)

Embryo transfer ©http://www.drmalpani.com/book/chapter25c.html (Accessed on 10/08/06, at 4:13pm) figure (4.2).

Victoria fertility centre ©http://www.victoriafertility.com/services/embryo-culture-transfer.htm(Accessed on 29/08/06 at 11.32 am) figure 4.3(a), and (b), figure 2.1(accessed on 16/06/07).

Anderson, R.E., Nugent, N.L., Gregg, A.T., Nunn, S.L., and Behr B. R. (2002) .Fertil.and Steril. Figures (4.4); 4.5(a); and 4.5(b).

Sahara and McClamrock (1997); Human Reproduction .figure 4.7(a) and (b)

Kunz, G., Beil, D., Huppert, P.,and Leyendecker, G. (2000). Human Reproduction. figure (5.1).

Noe, M. Kunz, G., Herbertz M., Mall, G. And Leyendecker, G. (1999).Human Reproduction. Figure (5.2).

Nakai, A., Togashi, K., Ueda, H., Yamaoka, T., Fujii, S., Konishi, J. (2001). J Women's Imaging; figure 5.3(a) and (b).

Dr Fanchin R. Hospital Antoine Beclere, France and Human Reproduction Figure 5.4(a),(b),(c). 5.5

## References

The references are listed in strict alphabetical order, including names beginning Mac and Mc, names including de are listed as de.

## Production

This thesis was typed on a Dell Optiplex GXa IBM compatible computer using Microsoft Word. The font used was Times New Roman 12. The text was printed using HP colour Laser Jet 2600n.

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## ABSTRACT OF THESIS

The overall purpose of this thesis was to identify factors which influence the outcome of IVF treatment and which might be amenable to modification in order to improve pregnancy rates following this form of treatment. To this end I performed a historical review of the advances that have been made in reproductive treatment since before the birth of Louise Brown in 1978, and a retrospective analysis of the result of a single IVF unit over a 6 year period from 1999 to 2005. This identified a number of factors on which IVF pregnancy rates depend.

I found that there is a decrease in the pregnancy and implantation rates and an increase in the abortion rates with a consequent decrease in the live birth rate with an increase in the female age. The data demonstrated that the pregnancy, implantation, abortion, and live birth rates for female age 20-25 years was 42%, 21.95%, 37.5%, and 26.3% respectively. For the age of 26-30 years the comparative figures were 44.5%, 27.4%, 12.34%, and 39%, for the age of 31-35 years 35.7%, 22%, 16%, and 29.9%, for the age of 36-39 years 27%, 13.9%, 22.7%, and 20.5% and for patients who were 40 years and above 17%, 7.4%, 27.3%, and 12.9%. (P<0.05) for the pregnancy, implantation, and live birth rates, (P>0.05) for the abortion rate. In addition, I found that there is a decrease in the pregnancy rate with increasing age of the male partner although this did not reach a statistically significant level. For male patients whose age was between 20-30 years the pregnancy rate was 42.7% compared with 37.8% if they were between 31-44 years and 31.6% if they were 45 years or above (P >0.05).

I found that the outcome of IVF-ET is affected by the number of embryos transferred, and whether the embryos are fresh or cryopreserved. The data showed that the pregnancy and implantation rates for single embryo transfer were 16.9%; for double embryo transfer were 37.3% and 23.22% respectively, and for triple embryo transfer 27.12% and 11.64% respectively. In addition, I found that the difference in the twin and triplet rates were 0% and 0% respectively for single embryo transfer, 24.5 and 0.02% respectively for double embryo transfer, and 26.8% and 1.5% respectively for triple embryo transfer. The differences in pregnancy and implantation rates between the transfer of two fresh and two frozen embryos were 37.3% versus 27.3% (P>0.05) and 23.22% versus 16.36% (P>0.05).

The outcome of IVF-ET was also found to be affected by the grade and cleaving rate of embryos. The differences in the pregnancy and implantation rates between the transfer of high-grade and low grade embryos were 61.4% versus 11.5%, and 35.8% versus 6.96% (P<0.0001). The differences in the pregnancy and implantation rates after the transfer of slowly cleaving embryos or rapidly cleaving embryos transferred on day 2 was 18.3% versus 44% respectively (P<0.0001), and 11% versus 28% respectively (P<0.0001). Similar differences were seen between slowly or rapidly cleaving embryos transferred on day 3. The difference were 20% versus 63.4% respectively (P<0.017) for the pregnancy rate, and 13.3% versus 40.14% respectively (P <0.027) for the implantation rate.

The day of embryo transfer was also relevant, with a better outcome when the embryos were transferred on day three rather than day two. The differences were 42.8% versus 35.1% (P<0.024) for the pregnancy rate and 27.43% versus 21.53% (P<0.005) for the implantation rate.

Transcervical embryo transfer (TCET) was more likely than transmyometrial embryo transfer (TMET) to lead to pregnancy, whether the transfer was easy or difficult. The outcome of TMET was low even if it was easy. Zygote intrafallopian transfer (ZIFT) is preferred to TMET if at least one fallopian tube is patent.

The outcome differs when different operators perform ET and the difference in the pregnancy rate for three different operators was found to vary between 35.2%, 41.2%, and 26.5% (P<0.026). The outcomes were good if nurses performed the procedure, and a new trainee was found to need around one year to become expert in the technique with pregnancy rates increasing from 34.54% to 47.3% at the end of one year of performing the procedures.

I found also that the outcome was affected by the cause of infertility, with better outcomes when the aetiology was tubal, unexplained, or polycystic ovary syndrome (PCOS), and poorer outcomes when the aetiology was endometriosis, untreated hydrosalpinges or after a history of ectopic pregnancy. Differences in pregnancy rate according to aetiology varied between 61.9% and 11.8% (P <0.0001).

By studying the results of the egg-sharing programme, I was able to show that aging of the ovary is more important than aging of the uterus and the outcome of IVF/ET in egg recipients is almost the same as the outcome in egg sharing donors. The pregnancy and implantation rates were 35.5%, and 18.33% respectively for egg recipients, and 35.5%, and 18.06% respectively for egg sharing donors. In addition, I found that abortion rates were higher and consequently live birth rates were considerably lower in the egg recipients as compared to the egg-sharing patients, abortion rates 26.19% versus 9%, $P>0.05$, live birth rates 26.5% versus 32.25%, $P>0.05$.

Finally, I found that abortion rates were higher and consequently live birth rates were lower with increasing age of recipient. The abortion and live birth rates according to the recipient age were 18.75%, 30.95% respectively for recipients less than 35 years, 28.57%, 26.31% respectively for those 36-39 years and 31.56%, 23.21% respectively for those 40 years and above, $P>0.05$. In addition, I found that the outcome is better when egg recipient patients have ovarian function as compared with egg recipient patients with no ovarian function, and the pregnancy rate was 41.3% for the first group and 29.62% for the second group, $P>0.05$. Egg sharing patients were found to have lower pregnancy and implantation rates while the live birth rate of egg sharing is virtually the same as standard IVF patients. The difference in the pregnancy, implantation, abortion, and live birth rates between the two groups was (35.52% versus 40.7%), (18.3% versus 25.61%), (9% versus 20.46%), and (32.25% versus 32.29%). Hence, egg sharing has no detrimental effect on the outcome for egg sharing patients.

The only factor amenable to modification for each and every couple was identified as the technique of embryo transfer. Hence, I undertook a literature search to identify the effects of the technique that might be relevant. I also used time-lapsed ultrasound video imaging of the uterus as a means of identifying those cycles that might have a favourable or unfavourable outcome as a result of a good or poor ET technique. My results show that exaggerated junctional zone contractions do indeed have a detrimental effect on the outcome of IVF-ET our data shows that the pregnancy rate for those who had less than 5 uterine contractions per 2 minutes as compared to the pregnancy rate for patients who had more than 5 uterine contractions per 2 minutes was

29.7% versus 0% respectively p=0.026, but an easy embryo transfer did not appear to change the character or the frequency of junctional zone contractions.

**To identify factors that influence the outcome of IVF treatment and which might be amenable to modification in order to improve IVF pregnancy rates.**

It was felt that even if relevant factors were identified they could only be tested in a large prospective randomised study of IVF pregnancy rates, which in many instances would be impractical. Therefore, a second aim was added.

**To identify ways in which the quality of any modification might be judged without the need for major clinical trials of IVF pregnancy rates**

In order to meet the first aim of the thesis the following methodology was employed

1   A historical review of the advances that have been made in the field of assisted reproduction

2   A retrospective analysis of the results of a single IVF unit (Hull IVF unit) for the last 6 years to identify factors on which the IVF pregnancy rate might depend, these factors included:
   Female and male age
   IVF or ICSI
   Embryo quality
   Embryo cryopreservation
   The number of embryos transferred in fresh and in frozen cycles
   Blastocyst transfer
   Day two or day three embryo transfer
   Easy or difficult embryo transfer
   Transcervical, transmyometrial or tubal embryo transfer
   Different operators performing embryo transfer
   Nurses performing embryo transfer
   New trainees performing embryo transfer
   Aetiology of infertility
   Treated and untreated hydrosalpinges
   Patients with or without a history of ectopic pregnancy

3   A retrospective analysis of the success of IVF in the egg-sharing program to investigate the following:
   Aging of the uterus versus aging of the ovary
   Egg recipients versus egg sharers of the same age group
   Age the egg recipients
   Egg sharers versus standard IVF patients

In order to meet the second aim of the thesis the following methodology was employed

4   A review of the literatures to identify effects of the technique of embryo transfer, which was found to be the only factor amenable to modification that might be relevant.

5    A study of the effects of junctional zone contractions on the outcome of IVF
     to determine if this could be used as a way of measuring the quality of embryo
     transfer

# CHAPTER ONE

## The History and Techniques of Assisted Reproduction

### 1.1 The history of assisted reproduction:

The announcement of the birth of Louise Brown on 25th of July 1978 ( Steptoe and Edwards 1978), see figure (1.1), was not the end of IVF development, but an important milestone along the way to what is now an important and internationally recognised treatment for many infertile women (Brinsden 1999). The first human embryo transfers had been attempted in 1972, and an illustration of the work and commitment that was involved is that these first 'experiments' at IVF and embryo transfer needed to be repeated 101 times before the first successful delivery was achieved (Edwards et al. 1980).



**Figure 1.1** Baby Louise Brown with Patrick Steptoe and Bob Edwards on 25th of July 1978, Delivered by LSCS at 39 weeks, her Weight was 2,608 gm
This figure is from BBC news (profile of Louise brown)
©http://news.bbc.co.uk/1/hi/health/3091241.stm
Accessed 14/08/06, 5.10 pm

There had been previous work on animals. A major discovery in the mid 18th century was that the egg would not develop without the sperm as a triggering factor for its development, and in 1840, Bischoft and Barry were the first who demonstrated spermatozoa penetrating the zona pellucida (Bischoft and Barry 1840). In the 1870s, Oscar Hertwig and Hermann Fol demonstrated the presence of two pronuclei in the cytoplasm of a fertilized Oocyte (Fishel and Symond 1986). Walter Heap (Heap1891) was responsible for the 1st successful embryo transfer in the rabbit in the late nineteenth

century, the embryo transfer having been performed between two species of rabbit, and the first successful IVF in animals, also in the rabbit, was subsequently achieved by Chang in 1959 (Chang 1959).

There were three major problems to achieve successful IVF in the human: the development of a suitable medium for the culture of the embryo (Kemeter and Feichtinger 1984), obtaining a suitable sperm with an acrosome reaction (Brinsden 1999), and obtaining an ovum ripe enough for fertilisation to occur (Charbonnel et al. 1987; Brinsden 1999).

Culture media have been developed gradually and their formulation has received intense attention. In 1949, Hammond, developed a complex medium that supported the growth of 8-cell mouse embryos to blastocysts (Hammonds 1949). Most embryo culture media used in early human IVF were based upon media that had been successfully used in animal embryo culture. The development of sequential tissue culture media, that attempt to give the growing embryo its metabolic and chemical needs, has resulted in an extension of the embryo culture period to the blastocyst stage, with higher implantation and pregnancy rates (Wilson et al. 2002).

Assisted reproductive techniques have existed for a very long time; the first recorded successful birth of a child following artificial insemination using the husband's semen was in London in 1785 (Bunge and Sherman 1953). Sperm capacitation was discovered in 1951. Freshly ejaculated or epididymal spermatozoa were found to be unable to fertilize ova in vitro, whereas spermatozoa that have resided in the oviduct of donor animals for 4–8 hrs were able to do so (Austin 1951; and Chang 1951).

White and Aitkin (1989) investigated the events associated with capacitation in the golden hamster. They suggested that the process was associated with a gradual increase in intracellular calcium, which in turn enhances the intracellular generation of cyclic AMP, and they suggested that in response to these changes the spermatozoa become hyperactive and primed to undergo the acrosome reaction. The same result was achieved by other studies (Fraser and Ahuja 1988; Jones et al 1990; and Aitkin 1990).

Much has been learnt about the need for sperm to undergo the acrosome reaction before fertilization and ways of inducing this artificially, either by exposing the

sperm in vitro to a pH of 8.6 (Aitkin 1990; and Bedford 1990) or to either progesterone or arachidonic acid (Pillai and Meizel 1991).

The first successful pregnancy following the use of frozen sperm was reported in 1953, the sperm had been stored at - 80°C using glycerol as a cryoprotectant agent (Bunge and Sherman1953). Then in 1964, the first pregnancy was recorded as a result of using sperm frozen in liquid nitrogen at -196°C rather than dry ice (Sherman 1963). Approaches to sperm cryopreservation tend to avoid the use of slow freezing technique used for the oocyte and the embryo; instead semi rapid methods are applied with a freezing rate of (-20°C) to (-30°C) (Edwards and Brody 1995). It was found that cryopreservation can damage the sperm acrosome, resulting in low levels of acrosin in surviving samples (Cross and Hanks 1991). The content of acetyl carnitine in spermatozoa has been shown to decrease during cryopreservation, which may impair subsequent motility, although the carnitine level will not be affected (Grizard et al. 1992).

Experiments have been performed on both animal and human oocytes with the initial stimulus to the introduction of human IVF arising from studies in mice. Pincus and Enzmann in 1935 were the first to show how oocytes of various animals would undergo maturation if released from their follicle and cultured in vitro (Cohen et al. 2005). The researchers attempted to do the same with human oocytes, predicting the maturation time to be 12 hours, as in mice. Others had shown that the follicle stimulant pregnant mares' serum gonadotrophin (PMSG) combined with hCG to induce ovulation would stimulate a large number of mouse oocytes to mature in the ovary. In addition, they found that each species had its own duration of maturation (Edwards 1956; Edwards and Gate 1959). By 1965 Edwards had established that the duration of maturation of human oocytes in vitro and he had showed conclusively that the interval between the LH surge or an injection of hCG would be approximately 37 hours ,history, 20th century –© www.repromed.org.uk/history/20th (accessed 22/08/06, 8.35 pm).

After 1954, human oocytes could be retrieved via ovarian biopsy, matured in vitro and used for studies on meiosis and fertilisation. In 1965, a human oocyte excised from an ovarian biopsy was observed through different stages of maturation by Edwards (Edwards 1965). After that, researchers tried not only to observe the

maturation of the oocyte in vitro but also to fertilize the oocyte in vitro (Edwards 1965; Steptoe and Edwards 1970).

In 1957, an extract of human pituitary containing both FSH and LH (Human Pituitary Gonadotrophin, hPG) was administered for ovulation induction. Until recently, it was not known that often these extracts caused incidences of Creutzfeldt-Jakob disease (CJD) more than 30years later (Gemzell et al. 1958). It was not until the 1960s that purified human menopausal gonadotrophin (hMG) and hCG were used Edwards et al. (1981), and "super ovulation" was first introduced in the late 1960s. It was utilised by Steptoe and Edwards (Steptoe and Edwards 1976), but they subsequently stopped using it as they thought that hMG had adversely affected the site of implantation of the first IVF pregnancy, which was ectopic (Edwards et al. 1981). In 1979, oocytes were harvested in the USA (Shettles 1979), and finally an IVF baby was delivered in Australia in 1980 (Lopata et al. 1980). The cycles were stimulated, and hMG was reintroduced into IVF (Jones et al. 1984).

Filicore and co-workers have emphasised that LH is necessary for oocyte maturation. Oocyte collection was timed by the administration of hCG (human chorionic gonadotrophin) 34–37 hrs before the expected time of ovulation (Filicore et al. 1999).

Another major problem in the early years concerned the method of oocyte collection. In 1966 laparotomy was the first method used to collect the oocyte (Edwards et al. 1981), although laparoscopy was quickly introduced in the late 1960s (Steptoe and Edwards 1970). Initially the pneumoperitonium was established with carbon dioxide; this was soon replaced by a mixture of 5% carbon dioxide, 5% oxygen, and 90% nitrogen to avoid acidic conditions in the abdomen and in the aspirated fluid (Edwards and Brody 1995). In 1982, oocyte collection was achieved by the use of abdominal ultrasound (Lenz and Lauritzen 1982; Hamberger 1982). Transvaginal ultrasound was introduced in 1983 (Gleicher et al. 1983), see figure (1.2).
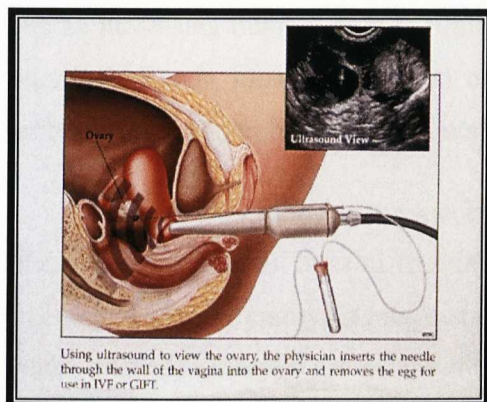
**Figure 1.2** Egg retrieval.
Under ultrasound guidance, oocyte will be aspirated by double lumen needle with the help of negative pressure; the follicular fluid will be aspirated to a plastic tube
Illustration from: In Vitro fertilization (Northern California Fertility centre)
©http://www.ncfmc.com/progsum.htm
(Accessed on 01/07/06 at 10:22 am)

Vaginal ultrasound Oocyte capture removes the need for women to have an uncomfortable full bladder during her ultrasound examination. Feichtinger and Kemeter (1984) found that ultrasonically guided follicular aspiration is superior to laparoscopic aspiration of the oocyte, and the success rate of this technique reached that of laparoscopic oocyte aspiration, in addition, they showed that the oocyte recovery rate was 93%, fertilization rate was 58%, and the pregnancy rate was 13%.
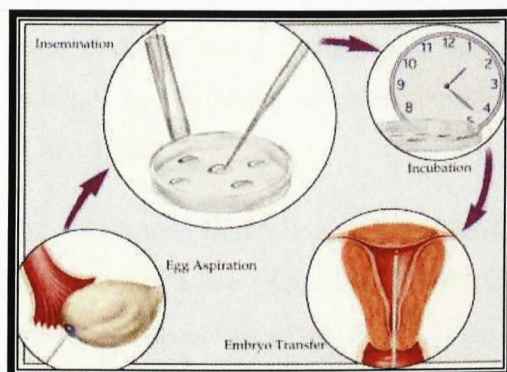


**Figure 1.3** IVF and ET procedure.
After ultrasound guided egg retrieval, the oocyte is inseminated and incubated for 16-18 hrs, following that fertilization is checked and embryo transfer performed after 48-72 hrs.
Illustration from In Vitro fertilization (Northern California Fertility centre)
©http://www.ncfmc.com/progsum.htm
(Accessed on 01/07/06 at 10:22 am)

Frozen embryo transfer is a well established form of assisted reproduction treatment. It allows multiple embryo transfers to be performed after single egg retrieval (Van Steirteghem et al. 1992). In 1953, Smith reported that exposure of the pre-implantation embryo to very low temperatures is not incompatible with further development, and the first frozen embryo transfer was performed in the rabbit by Chang and Morden in 1954 (Fishel and Symonds 1986). Twenty years later work was published on the success of embryo cryopreservation in the mouse (Whittingham and Wilmut1972). In the human, the first baby born after frozen embryo transfer was in 1984 at the Queen Victoria Hospital, Melbourne, Australia (Trounson and Mohr 1983),

and an increasing number of IVF clinics worldwide are now able to freeze embryos for later transfer. In most experienced centres, implantation rates using freeze–thawed embryos are comparable with implantation rates using fresh embryos.

Factors that influence the success of embryo cryopreservation include the choice of cryoprotectant used, rates of freezing and thawing, quality of embryos at the time of freezing, degree of embryo damage at the time of thawing, management of the cycle in which thawed embryos are replaced, timing of embryo transfer and the number of embryos transferred (Van Steirteghem et al. 1992; Van Steirteghem and Van den Abbeel 1993). The greatest danger to the cells lies in the formation of intracellular ice, risk can be reduced by very slow rate of freezing up to -6°C; cooling rate should be regulated after that at -1°C/min, and can be gradually increased as the temperature reaches -30°C or -40°C. The thawing rate should also be controlled (Ashwood and smith 1986).

Twins, a boy and a girl, were born in 1986, as a result of the use of frozen eggs, in Adelaide, Australia. Three of the mother's eggs were frozen at -196°C. The process was pioneered by Dr Christopher Chen, at the Flinders Medical Centre. Dr Chen suggested that using frozen eggs, rather than frozen embryos, removed some of the 'ethical, social, legal, moral and religious problems' associated with the technique (Chen 1986).

Gamete Intrafallopian Transfer (GIFT) was introduced in 1984, as an alternative to the standard IVF (Asch et al. 1984). GIFT and Zygote Intrafallopian Transfer (ZIFT) are related techniques, see figure (1.4), suitable for couples with at least one healthy fallopian tube (Devroey et al. 1986). In GIFT, oocytes and sperm are collected as for IVF-ET, but then both sets of gametes are combined and transferred immediately to the fallopian tube so that fertilization can occur in vivo. Up to three cumulus–oocyte complexes together with 100,000 progressively motile spermatozoa can be transferred by laparoscopy into the fimbrial end of the Fallopian tube at the time of the egg retrieval (Asch et al. 1984; Braeckmans et al. 1987), see figure (1.4). GIFT does not involve embryo culture. This enables some patients to pursue additional fertility treatments without addressing the ethical concern about how many embryos to create or transfer. Today attempts at fertilization by GIFT are rare. In 2001 they accounted for less than 1% of all attempts at fertilization used by ART patients, because of the

success rates of IVF [(The Regulation of New Biotechnologies, chapter two: Assisted reproduction)

**www.bioethics.gov/reports/reproductionandresponsibility/chapter2.html-243k**

(Accessed on 23/ 8/2006 at 5.10 pm)].
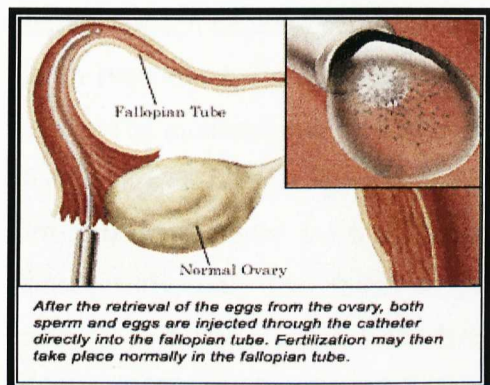


**Figure 1.4** GIFT. After retrieval of the eggs from the ovary both sperm and eggs are injected through the catheter directly in to the fallopian tube, fertilization may take place in Vivo Illustration from http://www.fertilityjourney.com/therapyOptions/assistedReproduction/artOverview/index.asp (Accessed on 02/03/06 at 2.53pm)

ZIFT is another IVF-ET variant, first used in 1986 (Devroey et al. 1986). An uncleaved "one cell with two pronuclei" or a cleaved fertilized oocyte is placed in the fallopian tube on the $1^{st}$ or $2^{nd}$ day after oocyte retrieval. The transfer can be done either orthograde with the use of laparoscopy, or retrograde through the uterus (Devroey et al. 1986; 1990). Pool et al, showed that pregnancy, implantation and delivery rates could reach 40%, 17%, and 34% in patients with long standing non tubal infertility, as compared with 32%, 11% and 21% with GIFT, and 26%, 8% and 16% with IVF (Pool et al. 1990).

Further attempts were made to facilitate fertilization by micromanipulation of the oocyte and the sperm, in order to treat couples with severe male factor infertility, who cannot be helped by conventional IVF. The idea is to remove the barrier to fertilization in vitro by disrupting the zona pellucida (Cohen et al. 1993).

The first technique of micromanipulation was partial zona dissection (PZD), in which a small slit is created in the zona pellucida in order to allow sperm to pass through to the membrane of the oocyte, see figure (1.6.A). The results of PZD were unsatisfactory because of low fertilization rates (Cohen et al. 1993), especially for men with a severe sperm defect, such as severe oligozoospermia, or low sperm motility.

Teratozospermia remained intractable and polyspermy was common (Tucker et al. 1991; Jean et al. 1992).

The next technique was subzonal sperm injection (SUZI), see figure (1.6.B). This is a technique by which one or several sperms (usually 3 to 20), are injected directly through the zona pellucida into the perivitelline space (Van Steirteghem 1993a), and in 1988, the first pregnancy was recorded using subzonal sperm injection (Ng et al. 1988). The monospermic fertilization rate using SUZI was still low, at about 20% of all metaphase II oocytes injected, and overall the experience with SUZI was that normal fertilization rates were too low (Fishel and Edwards 1983; Krzyminiska et al. 1992). Consequently, only two thirds of patients receiving this treatment ever produced embryos, such that pregnancy and live birth rates were unacceptably low (Imoedemhe and Sgue 1993; and Van Steirteghem et al 1994b).
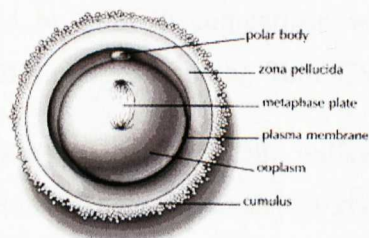


**Figure 1.5** A schematic illustration of the structural components of an oocyte those are important for micromanipulation. The oocyte is surrounded by three layers, first layer from outside is the cumulus, 2nd is the zona pellucida and the innermost layer is the oolemma. The presence of the first polar body indicates that this oocyte is mature and at Metaphase 2 Illustration from Schlegel and Girardi (1997). http://jcem.endojournals.org/cgi/content/full/82/3/709 Accessed 12/07/06, at 3:42pm
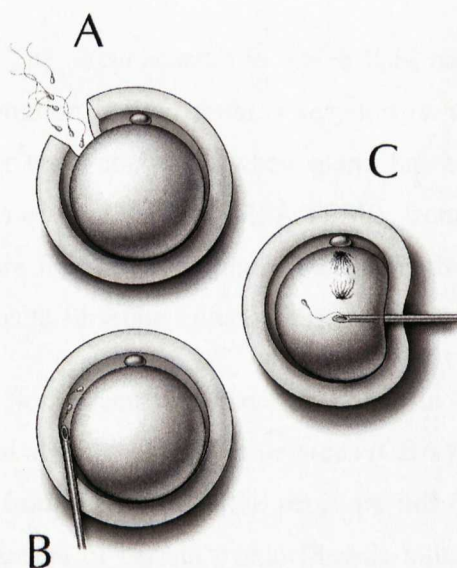


**Figure 1.6** Schematic illustrations of micromanipulation techniques.
**A.** Partial zona dissection (PZD).
**B.** Subzonal insertion (SUZI).
**C.** Intracytoplasmic sperm injection (ICSI).
Illustrations from Schlegel and Girardi (1997).
http://jcem.endojournals.org/cgi/content/full/82/3/709 Accessed 12/07/06, 3:42pm

ICSI was introduced in 1992, see figure (1.6.C), and figure (1.7). It was discovered accidentally, when, during attempted SUZI, a sperm was introduced in to the cytoplasm of the egg rather than in to the subzonal space. This egg was not destroyed but monitored for fertilization. When fertilization took place, a new chapter in assisted reproductive technology (ART) was opened (Palermo et al. 1995).

ICSI not only gives significantly better fertilisation rates than SUZI, but also produces more embryos with higher implantation rates (Van Steirteghem 1993a; 1993b). It has become the standard treatment for infertility caused by male factor, as sperm parameters do not appear to affect the outcome of this procedure, and the technique can be used successfully to treat couples who have too few sperms for conventional IVF (Van Steirteghem 1993b; Palermo et al. 1995). Thus, ICSI has been adopted as the technique of choice when assisted fertilization is required (Palermo et al 1995; Yang et al. 1996). It has been shown that similar fertilization rates can be achieved by ICSI in comparison with conventional IVF, when male factor is not involved; Embryos resulting from ICSI have an improved quality (Yang et al. 1996).

Fertilization by ICSI, unlike IVF, bypasses the normal plasma membrane interactions that have been shown to exclude foreign genes adhering to the spermatozoa. Brossfield et al. (1999) have demonstrated the tenacity with which exogenous human papillomavirus DNA binds to human spermatozoa, and that this virus remains adherent even after extensive sperm washing.

The circumstances in which ICSI may be appropriate include: when the sperm concentration or total count is very low, when the sperm cannot move properly, or are in other ways abnormal, when sperm has been retrieved directly from the epididymis (PESA) or the testicles (TESA/TESE), from the urine, or by electro ejaculation, when there are high levels of antisperm antibodies in the semen, or when there have been previous fertilisation failures (Yang et al. 1996).

Some men who have no sperm in their semen are found to have congenital bilateral absence of the vas deferens (CBAVD). In this condition, the tubes that carry sperm from the testes to the penis are missing. Two thirds of men with CBAVD are also carriers of certain cystic fibrosis mutations (Anguiano et al. 1992; Yang et al. 1996). Men with CBAVD and their partners may therefore wish to undergo genetic testing before proceeding with ICSI. Certain genes on the Y chromosome have been

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shown to be involved in the production of sperm, and deletion of these genes may be responsible for some men having few or no sperm in their semen. Micro deletions of sections of the Y chromosome may be found in 10-15 percent of men with azoospermia or severe oligospermia (Pryor et al. 1997). These micro deletions can be detected by polymerase chain reaction (PCR) and fluorescence in-situ hybridization (FISH) (Le Bourhis et al. 2000). Consequently, using sperm with such deletions to create an embryo might result in the same type of sub-fertility being passed from father to son (Kent-First et al. 1996).

Abnormal numbers or structures of chromosomes, particularly the sex Chromosomes (X and Y), may be associated with infertility in both men and women, and babies born from ICSI treatment may have a slightly increased risk of inheriting these abnormalities. Studies have found that up to 3.3% of fathers of ICSI babies have abnormal chromosomes, although it is estimated that up to 2.4% of the wider population have a chromosomal abnormality [Assisted conception program Intracytoplasmic Sperm Injection (ICSI) ©www.edinburghivf.org/icsi.html - 20k accessed 24/8/2006, 3.22 pm]

An important question debated about ICSI infants is the risk of congenital malformations, previous studies having reported similar rates of congenital malformations for both IVF pregnancies, and the general population 2.2% versus 3.3% (Alsalili et al. 1995). Kallen et al. (2005) did a retrospective study of 16,280 IVF children, 30% of them conceived after ICSI. They found that a 42% excess of congenital malformation, explainable by parental characteristics and in some cases by the high rate of multiple births. Among these children, 8% had a congenital malformation, and 5% had a relatively severe condition. An increase was seen for neural tube defects, choanal atresia and alimentary tract atresia. There was no difference in malformation rate according to IVF method except for an excess of hypospadias after ICSI.
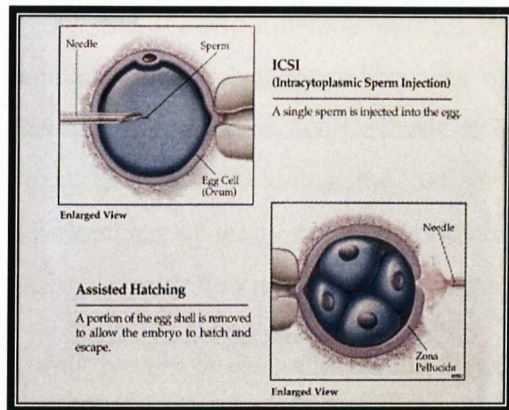
**Figure 1.7** Intracytoplasmic Sperm Injection (ICSI), and assisted hatching. The oocyte is stabilised under the microscope with a holding micropipette whilst the sperm is injected with an injection pipette.
Assisted hatching: a portion of the zona pellucida is removed to assist the embryo to hatch
Figure from In Vitro fertilization (Northern California Fertility centre ©http://www.ncfmc.com/progsum.htm Accessed on 01/07/06 at 10:22 am)

During ICSI, only mature oocytes are used for injection. The microinjection is performed with an injection pipette under the microscope equipped with a micromanipulator. After the oocyte is stabilised with a holding micropipette, the micropipette is pushed through the zona pellucida, and oolemma at the 3 o'clock position, keeping the polar body at 12 or 6 o'clock to avoid damage to the second meiotic spindle. The single sperm is injected headfirst (Palermo et al. 1993; 1995); see figure (1.7). Animal studies show that even with correct positioning, i.e. with the first polar body at right angles to the site of injection, spindle damage remains a theoretical risk, as the localisation of the first polar body does not invariably indicate the exact localisation of the spindle. Theoretically, the direct injection into the cytoplasm might induce chromosome breakage (Edirisinge et al. 1997), and another major concern is the introduction of contaminating foreign material into the oocyte cytoplasm, such as polyvinylpyrrolidone (PVP), which is used to slow down sperm movement, or a microorganism might act as contaminating vector (Brossfield et al. 1999).
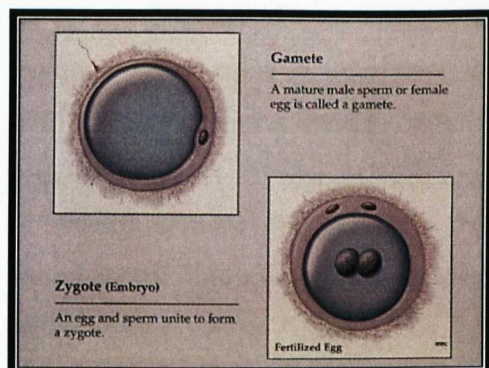


**Figure1.8** Gamete: A mature male sperm or female egg is termed a gamete

Zygote: an egg and sperm unite to form a zygote.
Illustrations from In Vitro fertilization (Northern California Fertility centre http://www.ncfmc.com/progsum.htm Accessed on 01/07/06 at 10:22 am

Steptoe and Edwards reported the first successful case of IVF in a 35 years old woman with tubal disease. The birth of Louis Brown was achieved by working in a natural cycle (Steptoe and Edwards 1978), but this birth was the culmination of many years of work, including the use of superovulation, which has now led to the development of many centres worldwide. The range of patients that are suitable for assisted conception is continually increasing.

In very large studies, found that the sex ratio for births resulting from ART does not differ significantly from the national ratios (Beral and Doyle 1990; FIVNAT "French In vitro National" 1995). Kausche et al. (2001) found that the sex ratio of infants born after blastocyst transfer was not significantly different from the sex ratio of births resulting from early cleavage stage embryo transfers, while Milki et al. (2003) found that male to female ratio is higher with blastocyst transfer compared to cleavage stage transfer. Moreover, a follow-up study of singleton infants conceived by ART has shown no difference in mental, motor, speech and social development compared to a matched control from the general population (Gibson et al. 1998). A similar result was found in a recent study by Ponjaert-Kristoffersen et al. (2005) in which children were recruited from five European countries. The study showed no difference in the motor and cognitive development of the children born after either ICSI or IVF as compared to that of normal conception children.

## 1.2 Factors affecting the pregnancy rate after IVF-ET

There is no reliable method to predict whether a particular embryo will implant and the process of implantation is almost inaccessible to ultrasound or any other method of inquiry. Techniques for raising the implantation rate after embryo transfer attract much discussion. Although the treatment of patients by assisted conception techniques has come a long way since 1978, much remains to be achieved. In particular, embryo implantation rates must be improved as up to 90% of all embryos transferred into the uterus still fail to implant (Edwards and Brody 1995). The success of assisted reproduction depends on the coordinated development of the embryo and the endometrium, and a persistent abnormality of either may lead to recurrent conception failure (Margalioth et al. 2006).

Although implantation is poorly understood in the human, the pregnancy rate following embryo transfer can be shown to be influenced by several defined variables.

Identifying variables in embryo transfer include embryo quality (Scoutt 2002), number of previous attempts, presence of blood or mucus on the transfer catheter (Nabi et al. 1997), age of the patient (Jansen 2003), number of embryos transferred (Allen et al 2006), and the cause of infertility( Edwards and Brody 1995).

In older studies, it was found that endometrial histological dating is usually of limited value (Cornilli et al. 1985), and endometrial morphology or cytochemistry is unable to predict the chance of implantation (Cornilli et al. 1985; Dockery et al 1990; Grudzinskas and Fay 1990). Kovachev et al. (2005) found that an endometrial volume of < 2 ml on the day of embryo transfer is a better predictor for low endometrial receptivity than endometrial thickness on the same day and resulted in significantly lower IVF clinical pregnancy and implantation rates. In addition, they found that an endometrial volume of > 2 ml on the day of ET was a positive predictor for ART outcome. Ng and co workers found that the vascularity of the endometrial and sub endometrial layers measured by 3D power Doppler Ultrasound is not a good predictor of pregnancy in FET cycles if measured at one time point only (Ng et al. 2006).

There may be relevant immunological factors, and transient or permanent expression of antiphospholipid antibody may give rise to impaired implantation in seropositive women, in whom heparin could be beneficial (Sher et al. 1994). Backos et al. (2002) emphasised that the increased prevalence of "antiphospholipid antibody" (apL) among women with infertility is likely to be part of a generalized autoimmune disturbance associated with infertility, and as it was found in other prospective studies that these antibodies do not significantly affect either the implantation or ongoing pregnancy rates. They concluded that routine screening for aPL among women undergoing IVF-ET is not warranted and therapeutic interventions should be used only in well designed randomized controlled trials.

The number of transferred embryos is an important variable influencing implantation after IVF. Not surprisingly pregnancy rates increase steadily as more embryos are replaced, but the higher implantation rate and live birth rate is outweighed by the risk of high order multiple gestation (Pandian et al. 2004).

Other factors which influence the implantation rate, are the quality of embryos, the rate of embryonic growth and the developmental stage i.e. cleavage or blastocyst stage (Wilson et al. 2002). Chromosomally normal embryos have a higher implantation

rate, so by doing pre implantation genetic screening and ensuring a chromosomally normal embryo in patients who have had implantation failure in the previous 3 cycles has been advocated (Cohen et al. 1990). Cohen has also claimed that assisted hatching, by artificial thinning of the zona pellucida, see figure (1.7), or by zona drilling, doubled the rate of implantation form 11% to 23 %, but the number of blighted ova and monozygotic twins might also increase. The main methods currently in use for assisted hatching are chemical, mechanical and laser. The process will damage about 1% of embryos. Most IVF clinics are not performing assisted hatching if there is one embryo available because of possible damage to this embryo would result in no embryo transfer IVF-Infertility©http://www.ivf-infertility.com/ivf/hatching.php (accessed on 16/06/07 at 1.32pm)

A deficient luteal phase could presumably impair the chances of implantation, but diagnosis of such a condition by endometrial biopsy in the previous cycle, is unreliable (Balasch et al. 1992a). Pritts and Atwood (2002) found that luteal supplementation with either i.m. hCG or i.m. progesterone significantly improved fertility outcomes compared with no treatment.

In the UK, IVF clinics are regulated by the Human Fertilisation and Embryology Authority (HFEA), which is an independent regulator. The HFEA was established to license, monitor and regulate clinics performing IVF and treatments using human sperm, eggs and embryos, as well as to promote safe and appropriate practice in fertility treatment and embryo research. The HFEA also regulates the storage of gametes (eggs and sperm) and embryos. It was established under the Human Fertilisation and Embryology Act 1990 (HFEA 2004). The HFEA gives advice and information to patients, clinics and doctors; it publishes a *'Code of Practice'* for centres carrying out licensed treatment. Guidelines in the *'Code of Practice'* consider: the respect which is due to human life at all stages in its development and the right of people who are, or may be infertile to proper consideration of their request for treatment [HFEA and GMC 2004 http://www.gmc-uk.org/about/partners/human_fertilisation_and_embryology_authority.asp accessed 24/08/06 at 2:12 pm]

## *1.3 Conclusions:*

"To understand science it is necessary to know its history" [Auguste Comte 1798-1857 ©http://www.bolender.com/Sociological%20Theory/Comte,%20Auguste/comte,_auguste.htm accessed on 25/09/06 at 3.45pm]. The importance of in vitro fertilization (IVF) as a treatment option has become apparent at a time when there is increasing recognition that many previously used treatments are ineffective, or less effective. However, IVF is not effective in all circumstances. The efficacy of IVF can only be accepted if treatment can be shown to do better, in defined circumstances, than other comparative treatments. Treatment still strongly depends on many variables, and in order to illustrate these variables I performed a retrospective study, trying to show the relationship between numerous variables identified in the literature review of this chapter and the local success rate of IVF. The aim was to go on to make recommendations for changes in local practice that might optimise success rates. One point I want to highlight, is that before the advent of intracytoplasmic sperm injection (ICSI), male infertility was recognized to significantly comprise the outcome of IVF treatment. ICSI treatment has revolutionized the treatment of the male, by making assisted reproductive techniques available to this group of infertile couples. The adaptation of ICSI encouraged the introduction of surgical sperm recovery techniques, which have further increased the options in this area.

# CHAPTER TWO

# A Retrospective Analysis of Patient Records in Order to Identify Factors Predictive of Successful IVF/ET Treatment

## 2.1 The aim of the study:

The purpose of this study was to identify factors positively and negatively correlated with pregnancy outcome after IVF and ET.

The factors which were analysed in this study were derived from the literature search into the history and techniques of IVF as described in chapter 1. They were as follows

1. The difference between the outcome of IVF and ICSI
2. The effect of female and male age.
3. The effect of the number of embryos transferred in fresh or in frozen cycles
4. The effect of embryo cryopreservation on the outcome of IVF
5. The effect of the grade and number of blastomeres of the transferred embryos
6. Day 2 versus day 3 embryo transfer
7. The outcome of blastocyst transfer
8. The effect of the aetiology of infertility
9. Differences in the outcome between TCET (easy or difficult), TMET or ZIFT.
10. Differences in the outcome between different operators
11. The outcome if nurses perform the embryo transfer procedure
12. The outcome if a new trainee were to start the embryo transfer procedure

## 2.2 The Type of study:

This study was a retrospective analysis of clinical notes. The setting was a single academic research centre based at the Hull IVF unit. The unit has been providing IVF treatment for 20 years, performing between 200 and 300 cycles per year, and has complete records of all treatment cycles performed.

## 2.3 Patients:

The population studied was 773 patients who had undergone 1629 IVF treatment cycles between 1$^{st}$ Jan 1999 and 31$^{st}$ Dec 2005. The notes were accessed alphabetically so as to include all the patients who had had IVF and IVF/ICSI during this period and all cycles were included.

## 2.4 Method:

A total of 1629 IVF-ET cycles were analysed retrospectively in 773 patients including 1292 fresh cycles, and 101 frozen cycles. The fresh cycles had all been treated with the same long protocol. I excluded cycles which were treated with citrotide Complete pituitary desensitization was achieved with a GnRH agonist, most of the patients had subcutaneous Buserelin, (Superfact, Aventis Pharma Deutschland GmbH, Bruningstrasse 50, Frankfurt am Main, Germany) 1mg subcutaneously daily starting on day 21 to 25 of the cycle according to individual cycle length. Initially a few patients were treated with nasal Nafarelin but because of the inconvenience of nasal administration the Hull IVF unit changed its policy to use Buserelin. The duration of drug treatment was until complete ovarian suppression was achieved (endometrium less than 4mm and/or serum oestradiol less than 100pmol/l).

Mock embryo transfer was introduced as unit policy in 1999, and hence mock embryo transfer was performed for the vast majority of the patients who are included in the study, following a scan to confirm down regulation. Ovarian hyperstimulation was then started and individually adjusted according to the speed and number of follicular growth. The starting dose was 150-225 IU of urofollitrophin (Metrodin high purity: Serono Laboratories UK Ltd, Welwyn Garden City, UK). Monitoring of the follicular

growth was performed every other day after the 5th day of stimulation, until the lead follicle reached a diameter of 18-20mm, when 10,000 IU hCG (Profasi, Serono) was given as an ovulatory trigger. Oocytes were harvested by transvaginal ultrasound guided puncture at approximately 36 hrs after hCG administration. All patients received 600mg of Ibuprofen (Brufen; Knoll Ltd, Nottingham, UK) 2 hrs before oocyte retrieval, which was performed under i.v. sedation using Midazolam (Antigen International Limited, Roscrea, co. Tipperary, Ireland), and Alfantanil (Rapifen; Janssen-Cilag Ltd, High Wycombe, Buck, UK). The partners gave their semen on the day of egg retrieval, in the lab the sample was washed out from the dead cells and seminal fluid. Insemination was performed 4-6 hrs after preparation of the semen. 75,000-100,000 sperm / oocyte were used in IVF cases while in ICSI cases one sperm was used per oocyte. Insemination was performed 4-6 hrs later and fertilization checked after ≈ 18-20hrs. Cleavage was checked after 44-48hrs and embryos were graded according to their morphology, degree of cytoplasmic fragmentations and cleavage stage. A scale was used from grade 1 to grade 5, with grade 5 regarded as the best embryo. Embryos were regarded as grade 5, if there was no cytoplasmic fragmentation; the blastomeres were equal in size and regular in shape. When the embryos were slightly fragmented (i.e. the fragmentation were less than 20%), they were regarded as grade 4. Embryos with 20-50% fragmentation were regarded as grade 3, while moderately fragmented embryos (i.e. embryos with more than 50% fragmentation) were regarded as grade 2 and severely fragmented embryo was regarded as grade 1.see figure 2.1(a),(b),(c),(d),(e). Any embryo that was equal to or above grade 3 was regarded as a good embryo. Embryo transfer was timed according to the day of egg retrieval, with patients who had egg retrieval on a Monday or a Wednesday having embryo transfer 48 hours later, whereas patients who had egg retrieval on a Friday had their embryo transfer 72 hours after egg retrieval. All patients had progesterone oral tablets for luteal phase support starting on the day of egg retrieval, which was changed to vaginal micronised progesterone (Utrogestan, Besin, Iscovesco, laboratories, Paris, France) after embryo transfer. Luteal support was continued for 2 weeks until the result of the pregnancy test was known. The three outcomes studied were pregnancy rate, implantation rate abortion rate and live birth rate.

Data were analysed on Statistics Package for Social Sciences for Windows (SPSS UK Ltd, St. Andrews House, Woking, and Surrey, UK) using logistic regression and chi square testing.



(a)                    (b)

(c)                    (d)                    (e)
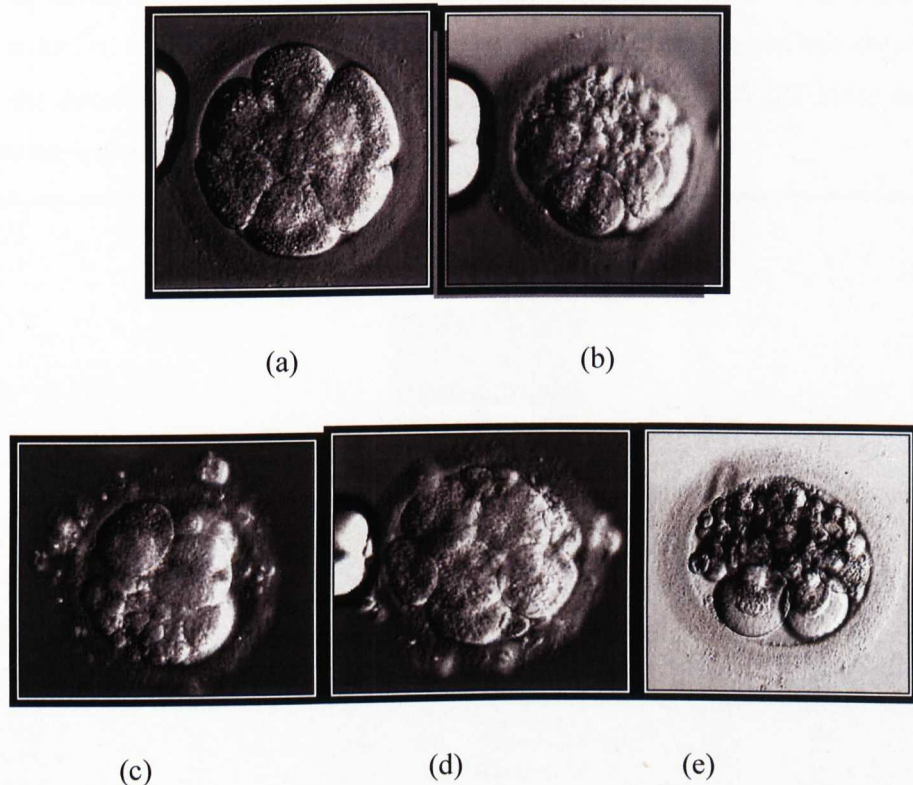
**Figure 2.1** Grade of the embryos, (a) grade 5 embryo, the cytoplasm is clear and the blastomeres are regular and equal in shape, (b) grade 4 embryo there is <20% fragmentation, (c) grade 3 embryo there is 20-50% fragmentation, (d) grade 2 embryo, the fragmentation is >50% but its moderate, (e) grade 1 embryo the fragmentation is sever. [This figure is from©www.advancedfertility.com/embryoquality. accessed on 18/06/07]

## 2.5   Results of the Study

A total of 773 patients had 1629 IVF cycles, of which 233 cycles (14.3%) were cancelled. These cancelled cycles included 51 cycles (3.13%) abandoned because of ovarian hyperstimulation syndrome (OHSS). All of the cases of OHSS were mild to moderate, except for two patients who had severe OHSS and had to be admitted for treatment of a pleural effusion and ascites.  123 cycles (7.5%) were abandoned due to under stimulation, 1 cycle was abandoned due to activation of endometriosis, 1 cycle

was abandoned due to development of a haemorrhagic ovarian cyst, 3 cycles were abandoned due to no sperm following TESE, 4 cycles were abandoned due to no egg being collected and 44 cycles (2.7%) were abandoned after egg retrieval (42 due to failure of fertilization and 2 due to late cleavage). A further 5 cycles was abandoned due to failure of frozen embryos to survive after thawing and one cycle was abandoned due to the development of uterine bleeding. Hence a total of 1393 (85.51%) embryo transfers were analysed see figure 2.2.
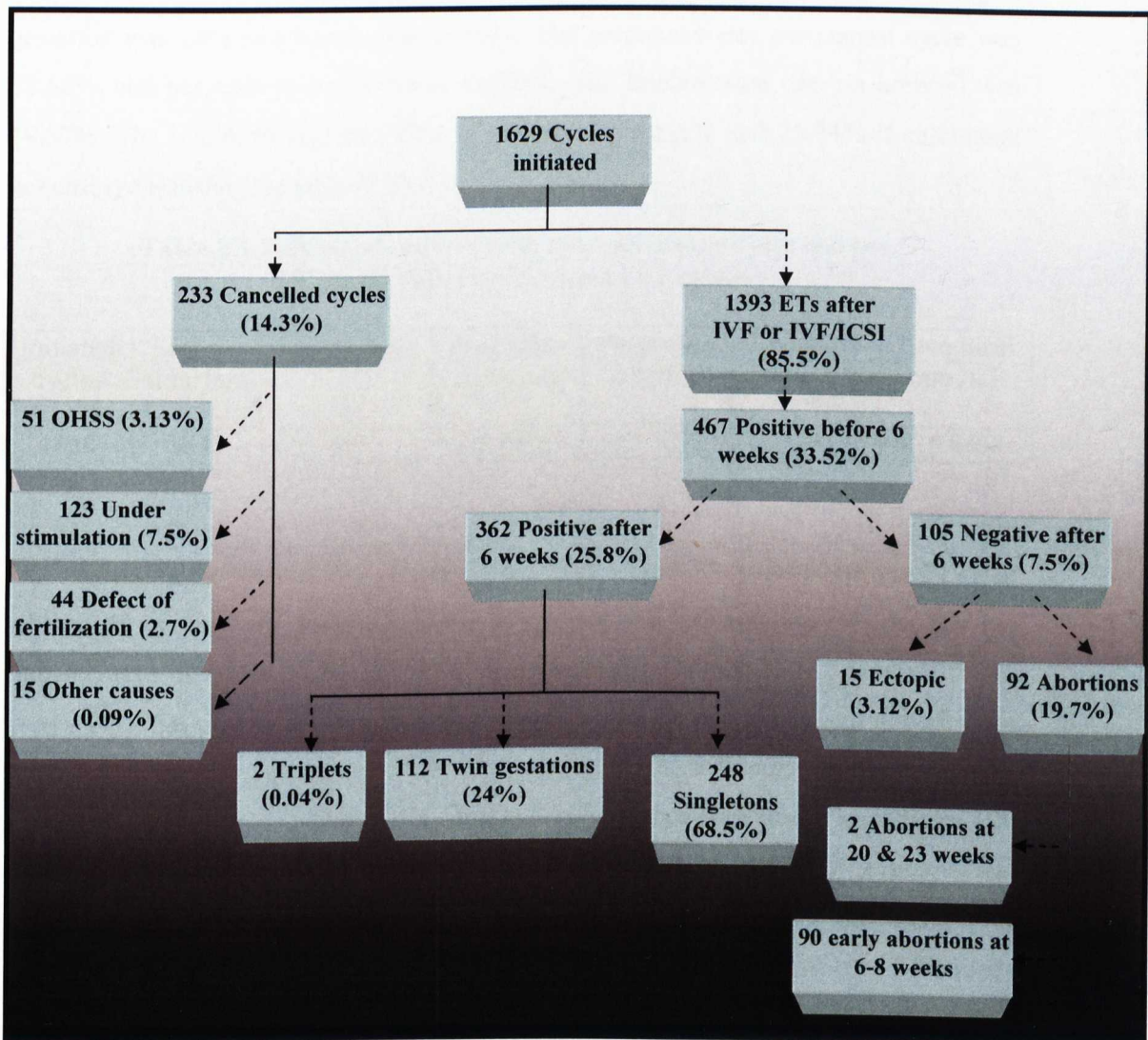


**Figure 2.2** The outcome of total IVF and IVF/ICSI cycles in the retrospective study including fresh and frozen cycles. This figure shows the number of initiated cycles, cancelled cycles, positive pregnancy tests before and after 6 weeks, number of ongoing pregnancies, incidence of single, twin and triplet, incidence of abortion and ectopic in the retrospective study.

Of the 1393 completed cycles, 467 cycles (33.52%) produced positive pregnancy tests before 6 weeks, and 362 cycles (25.8%) remained positive after 6 weeks. 92 cycles ended in abortion showing that the risk of abortion is 19.7%. In 90 cycles the abortion was early in the first 6-8 weeks, whereas 2 abortions were late, one at 20 weeks and one at 23 weeks. 15 cycles produced an ectopic pregnancy with the risk of ectopic pregnancy therefore being 3.12%. 112 cycles produced a twin gestation and 2 cycles produced a triplet gestation, therefore the incidence of twin and triplet gestation was 24% and 0.04% respectively. The pregnancy rate per started cycle was 28.66%, and per embryo transfer was 33.52%. The implantation rate per embryo was 19.37%. The live birth rate was 22% if calculated per cycle and 25.84% if calculated per embryo transfer, see table (2.1).

**Table 2.1** Pregnancy and live birth rates per started cycle and per ET for all IVF, IVF/ICSI and FET cycles

| Initiated cycles | Embryo transfers | Pregnancies | Pregnancy rate/cycle | Pregnancy rate/ET | Live birth rate/cycle | Live birth rate /ET |
|---|---|---|---|---|---|---|
| 1629 | 1393 | 467 | 28.66% | 33.52% | 22% | 25.84% |

Among the total of 1292 fresh cycles, there were 830 cycles (64.2%) of IVF of which 288 cycles were positive and the pregnancy and implantation rate per embryo transfer were therefore 34.7% and 20.53% respectively. The other 462 cycles (35.8%) were IVF/ICSI, of which 155 cycles were positive and the pregnancy and implantation rates were therefore 33.5% and 18.84% respectively. The difference in the pregnancy and implantation rates between IVF and IVF/ICSI is not statistically significant [P= 0.688 (> 0.05), odds ratio (OR) and 95% CI 0.952 (0.749-1.210)] for the pregnancy rate, and [P = 0.289 (>0.05), OR and 95% CI 0.899 (0.738-1.095)] for the implantation rate, see table 2.2. Limiting the figures to fresh cycles with the transfer of only 2 embryos there were 621 IVF cycles, with 235 cycles producing positive results, and 351 cycles of IVF/ICSI, of which 128 cycles were positive. The pregnancy and implantation rates for IVF were therefore 37.8% and 24.35%, and for IVF/ICSI the values were 36.5% and 21.36% respectively [P = 0.670 (> 0.05) OR and 95% CI 0.943 (0.719-1.237)] for the pregnancy rate, and [P= 0.134, OR and 95% CI 0.844 (0.676-1.054)] for the

implantation rate, see table 2.3 and figure 2.3. This shows that there is no statistically significant difference between the outcome of IVF and the outcome of IVF/ICSI.

**Table 2.2** Differences in the outcome between fresh IVF and fresh IVF/ICSI cycles. All cycles included except for FET cycles

|  | TOTAL fresh | IVF | ICSI | P | Odds ratio & (95% CI) |
|---|---|---|---|---|---|
|  | 1292 | 830 (64.2%) | 462 (35.8%) |  |  |
| Pregnancies | 443 | 288 | 155 |  |  |
| Pregnancy rate / ET | 34.3% | 34.7% | 33.5% | 0.688 ie. P> 0.05 | 0.952 (0.749-1.210) |
| Implantation rate/embryos | 19.94% | 20.53% | 18.84% | 0.289 ie. P>0.05 | 0.899 (0.738-1.095) |

**Table 2.3** Differences in the outcome between IVF and IVF/ICSI cycles. Only cycles in which two fresh embryos were transferred included.

|  | TOTAL | IVF | ICSI | P | Odds ratio & 95% CI |
|---|---|---|---|---|---|
|  | 972 | 621 | 351 |  |  |
| Pregnancies | 363 | 235 | 128 |  |  |
| Pregnancy rate / ET | 37.3% | 37.8% | 36.5% | 0.670 ie. P> 0.05 | 0.943 (0.719-1.237) |
| Implantation rate/embryos | 23.19% | 24.35% | 21.36% | 0.134 ie. P> 0.05 | 0.844 (0.676-1.054) |
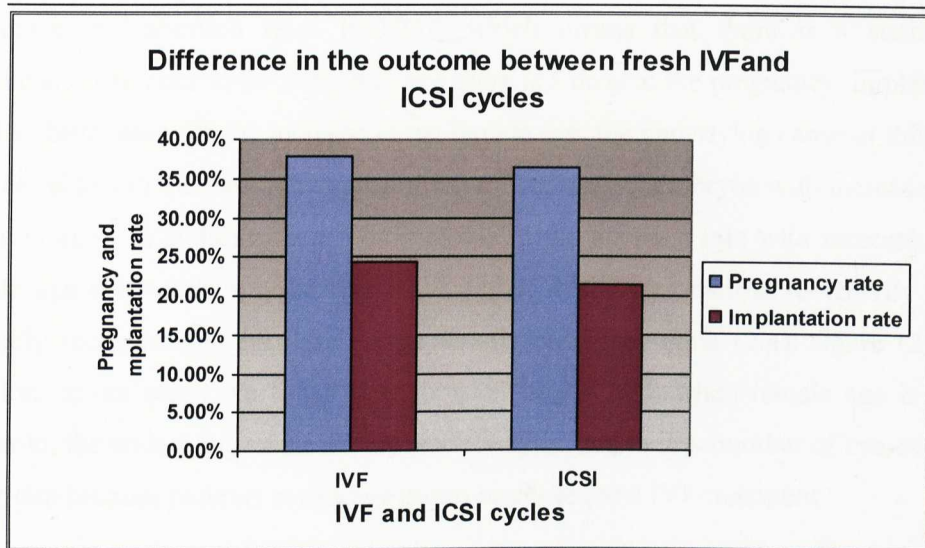
**Figure 2.3** Differences in the outcome between fresh IVF and fresh IVF/ICSI cycles. Differences in the pregnancy and implantation rates are not statistically significant.

## 2.5.1 Effect of the female age on the outcome of IVF/ET:

In our study, there were 830 fresh IVF cycles in which there was no male factor infertility according to WHO criteria (WHO 1992). Of these, 19 cycles (2.3%) featured a female with an age between 20-25yrs and of these, 8 cycles were positive and the pregnancy, implantation, abortion, and live birth rates were (42.1%, 21.95%, 37.5%, and 26.3% respectively). 182 cycles (22%) were for females whose age were 26 – 30 yrs and of these 81 cycles were positive and the pregnancy, implantation, abortion, and live birth rates were (44.5%, 27.41%, 12.34%, and 39% respectively). In 401(48.3%) cycles the female age was 31-35 with 143 cycles positive and the pregnancy, implantation, abortion, and live birth rates were (35.7%, 22%, 16%, and 29.9% respectively). 166 cycles (20%) the female age was 36 – 39, and 45 cycles were positive and the pregnancy, implantation, abortion, and live birth rates were therefore 27%, 13.9%, 22.7%, and 20.5% respectively). In 62 cycles female age was 40 or above and 9 cycles were positive with a pregnancy rate of (17%, 7.4%, 27.3%, and 12.9% respectively). P value, Odds ratio, and 95% CI for the difference in pregnancy, implantation and live birth rates were [P < 0.0001, Odds ratio and 95% CI were equal to 1.092 (0.420-2.841)], [P<0.0001, Odds ratio, and 95% CI 1.343(0.620-2.909)], and [P<0.0001, Odds ratio, and 95% CI 1.791(0.618-5.188)] respectively, P value for the

difference in abortion rates P=0.237, which means that there is a statistically significant difference in the outcome, and there is a drop in the pregnancy, implantation, and live birth rate with the increase in the female age, the underlying cause of this result may be related to increase in the proportion of low quality embryos with increase in the maternal age. In addition there was increase in the abortion rate with increase in the female age this result may be related to defect in the endometrial receptivity which probably increase with increase in the female age, see table (2.4), figure (2.4). In addition, in our study we found that abortion rate is high when female age is 20-25 years old, the underlying cause of this result may be due to low number of cycles at this age group because patients at this age group rarely request IVF treatment.

**Table 2.4** Effect of the female age on the outcome of IVF-ET. All the cycles were fresh IVF cycles, and no male factor infertility. Difference in the pregnancy, implantation, and live birth rates were checked with Chi square, and logistic regression tests.

| | Female age 20-25 yrs | Female age 26-30 yrs | Female age 31- 35 yrs | Female age 36 –39 yrs | Female age $\geq 40$ yrs | P value, Odds ratio, and 95% CI |
|---|---|---|---|---|---|---|
| Total | 19 (2.3%) | 182 (22%) | 401 (48.3%) | 166 (20%) | 62 (7.5%) | |
| Pregnancies | 8 | 81 | 143 | 45 | 9 | |
| Pregnancy rate / ET | 42.1% | 44.5% | 35.7% | 27% | 17% | P < 0.0001 OR and 95% CI 1.092 (0.420-2.841) |
| Implantation rate/ embryo | 21.95% | 27.41% | 22% | 13.9% | 7.4% | P<0.0001 OR and 95% CI 1.343 (0.620-2.909) |
| Abortion rate | 37.5% | 12.34% | 16% | 22.7% | 27.3% | P = 0.237 |
| Live birth rate | 26.3% | 39% | 29.9% | 20.5% | 12.9% | P<0.0001 OR and 95% CI 1.791 (0.618-5.188) |
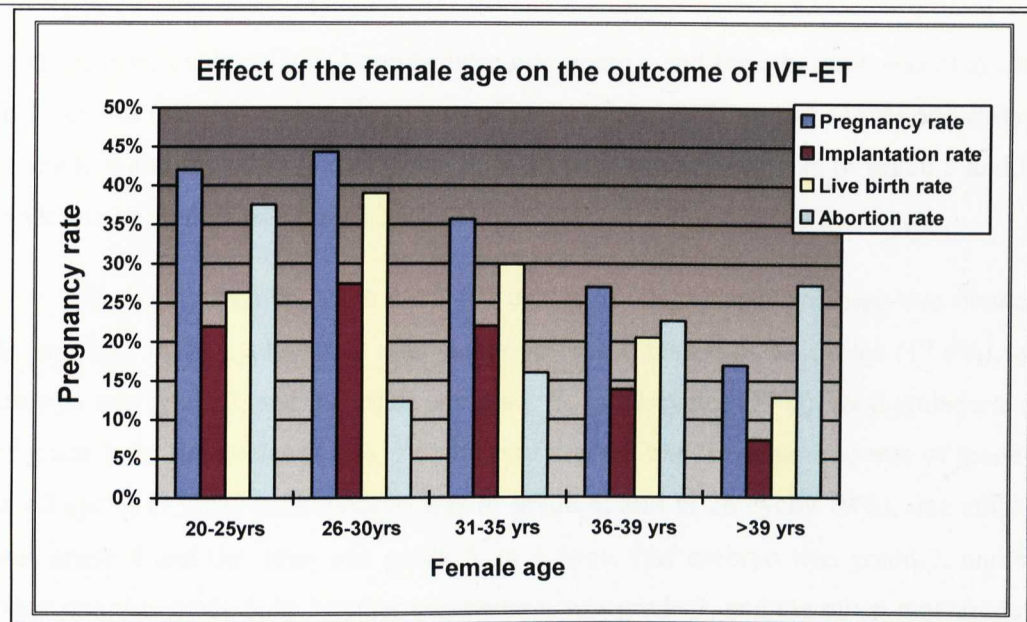
**Figure 2.4** Effect of female age on the outcome of IVF-ET. There is a decrease in the pregnancy, implantation, and live birth rates with increasing female age.
In addition there is an increase in the abortion rate with increasing female age

## 2.5.2 Relation between female age and the grade of embryos:

In our study, there were 830 fresh cycles of IVF that featured no male factor infertility and it was shown that the distribution of the grade of embryos according to female age varied as following: in 19 cycles the female age was between (20-25 years). Of these, there was 1 cycle in which there was a single embryo transfer, 4 cycles of triple embryo transfer and the remaining 14 cycles were double embryo transfers. In 3 cycles (21.4%), one embryo was grade 2 and the other one was grade 3, in 2 cycles (14.3%) both embryos were of grade 3, in 7 cycles (50%) one embryo was grade 3 and the other one was grade 4, in 2 cycles (14.3%) one embryo was grade 4 and the other one was grade 5, see table (2.5).

The distribution of the embryos in the second group, which consisted of 182 cycles in which the female age was between 26 and 30, was as follows: there were 4 cycles in which there was single embryo transfer; 22 cycles of triple embryo transfer and the remaining cycles were of double embryo transfer. In 14 cycles (9%) one embryo was grade 2 and the other grade 3; in 34 cycles (22%) both embryos were of

grade 3; in 68 cycles (43.6%) one embryo was grade 4 and the other one was of grade 3; in 19 cycles (12.3%) each embryo was of grade 4; and in 20 cycles (13%) one embryo of grade 4 and the other one of grade 5; in 1 cycle one embryo was of grade 3 and the other one of grade 5. see table (2.5).

In the third group, there were 401 cycles in which the female age was between 31 and 35. In 22 cycles there was single embryo transfer; in 54 cycles (17.6%), one embryo was grade 2, and the other one grade 3; in 83 cycles (27%), each embryo were of grade 3; in 100 cycles (32%), 1 embryo of grade 3 and the other one was of grade 4; in 42 cycles (13.5%) each embryo was of grade 4; and in 28 cycles (9%), one embryo was grade 4 and the other one grade 5; in 1 cycle one embryo was grade 2, and the other one was grade 5; in 3 cycles one embryo was grade 3, and the other was grade 5; in 1 cycle each embryo was of grade 5; and in 67 cycles there were triple embryos transferred, see table (2.5).

In the fourth group, of 166 cycles, in which female age was 36-39, 5 cycles had single embryo transfer; in 20 cycles (16.5%), two embryo transfer, one embryo was of grade 2, and the other was of grade 3; in 30 cycles (31.3%), both embryos were of grade 3; in 41 cycles (38%), one embryo of grade 3, and the other one was of grade 4; in 9 cycles (9.2%) had embryos that were each of grade 4; in 10 cycles (10.2%), one embryo was of grade 4 and the other was of grade 5, and in 2 cycles, one embryo of grade 3, and the other one was of grade 5; in 1 cycle there were transfer of 2 fresh blastocyst; and in 48 cycles there were triple embryo transfer see table (2.5).

In the last group of patients 62 cycles (female age was equal or more than 40). In 6 cycles, there was single embryo transferred, in 29 cycles, there were triple embryos transferred. In 1 cycle, both embryos were of grade 2, in 6 cycles, 1 embryo was of grade 2 and the other was of grade 3 (22.2%). In 10 cycles (37%) both embryos were of grade 3, in 9 cycles (33.3%), 1 embryo was of grade 3 and the other was of grade 4, and in 1 cycle (3.7%), 1 embryo was of grade 4 and the other 1 was of grade 5 see; table (2.5).

**Table 2.5** Proportion of the grade of embryos according to female age in all fresh IVF cycles, in which 2 embryos were transferred and no male factor infertility

| Grade of the embryos | Female age 20-25 yrs (19) | Female age 26-30 yrs (182) | Female age 31-35 yrs (401) | Female age 36-39 yrs (166) | Female age >39yrs (62) |
|---|---|---|---|---|---|
| 2 embryos each grade 2 | ------- | ------- | -------- | ------- | 1 1.6% |
| One embryo grade 2 and one grade 3 | 3 21.42% | 14 9% | 54 17.6% | 20 16.5% | 6 22.2% |
| 2 embryos, each grade 3 | 2 14.3% | 34 22 % | 83 27% | 30 31.3% | 10 37% |
| One embryo grade 3 and one grade 4 | 7 50% | 68 43.6% | 100 32% | 41 38% | 9 33.3% |
| 2 embryos, each grade 4 | ---- | 19 12.3% | 42 13.5% | 9 9.2% | ---- |
| One embryo grade 4 and one grade 5 | 2 14.3% | 20 13% | 28 9% | 10 10.2% | 1 3.7% |
| One embryo grade 3 and one grade 5 | - ------- | 1 | 3 | 2 | ------ |
| One embryo grade 2 and one grade 5 | ------- | ------- | 1 | ------- | ------- |
| 2 embryos each grade 5 | ------ | -------- | 1 | -------- | ------- |
| 2 fresh blastocysts | --------- | --------- | -------- | 1 | ------- |

From the previous table we can see that more than 60% of embryos within each age group were of grade (3,3) or (3,4), see figure (2.1) .The distribution of the low grade embryos i.e. one embryo of grade 2 and the other one of grade 3, were highest in the older age group (i.e. female age 40 and above), and we found that 22.2% of the embryos in this age group were of low grade, this probably explain the decrease in the pregnancy rate with increasing female age. In addition, we found the proportion of low grade embryos was lowest in the age group 26-30yrs, this may partly explain the highest pregnancy rate in this age group, while at the age of 20-25yrs we had only 19cycles, because patients rarely requesting IVF treatment at this

age, with higher number of patients at this age group the picture may be changed see table (2.5), and figure (2.5). For high grade embryos the distribution is highest in the young age group and it gradually decreases as the patient become older and is lowest in the old age group i.e. patients of 40 years and above, at which the percentage is low 3.7%, P= 0.087, see figure (2.5). Further, more we found that in all age groups around 75% of embryos are of grade 3 and above and according to our lab, embryos of grade 3 and above are regarded as good quality embryos, we can also see that after the age of 35 years old there is a reduction in the pregnancy, implantation, and live birth rates in spite of good quality embryos which suggests that the defect may be in the chromosomes and therefore cannot be detected.



**Effect of the female age on the grade of the embryo**
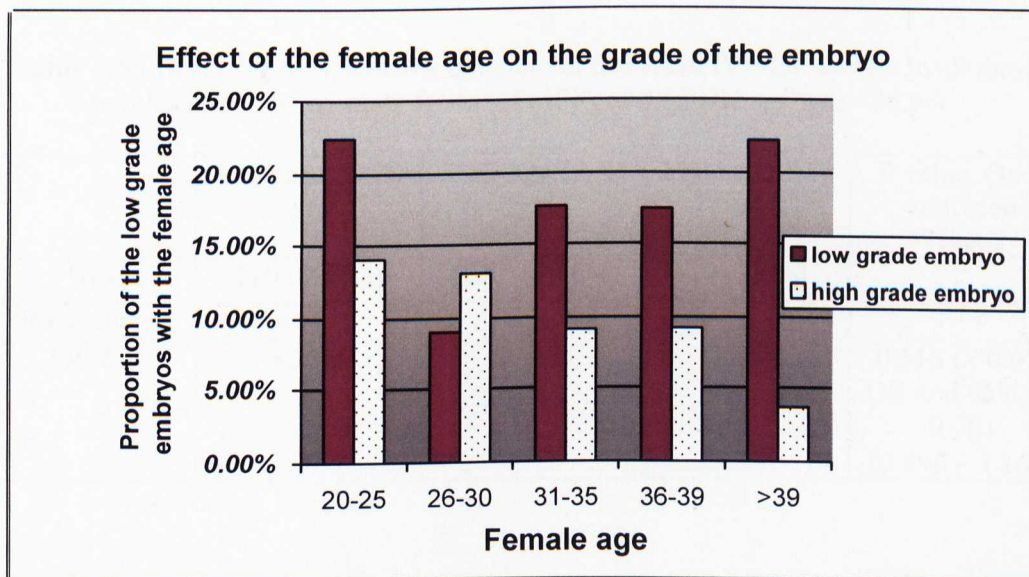
**Figure 2.5** Proportion of high grade and low grade embryos according to female age. This figure shows that there is step wise decrease of the percentage of high grade embryos with the increase in female age; in addition it shows that the lowest proportion of the low grade embryos were among the cycles in which the female age is between 26-30 years. This may partly explain the highest pregnancy rate in this age group.

## 2.5.3 Effect of the male age on the outcome of IVF-ET:

The age distribution in 1393 cycles with regard to male age was as follows: there were 110 cycles (18.2%) in which male age was between 20-30 years. In these cycles there was no male factor infertility according to WHO criteria (WHO 1992), and all the cycles were fresh, and the female age was equal to or below 35. Of these, 47 cycles were positive and the pregnancy rate was 42.7%. In 474 cycles (78.6%) the male age was between 31-44 years. Of these, 179 cycles were positive and the pregnancy rate was 37.8%. In 19 cycles (3.2%) the male age was equal to or above 45 and the female age was equal to or below 35 and of these, 6 cycles were positive and the pregnancy rate was 31.6%. [P= 0.516 (>0.05), and 95% CI = 0.761 (0.496 – 1.168)]. We could not find any significant effect of male age on the outcome of IVF-ET, see table (2.6), and figure (2.6).

**Table 2.6** Effect of male age on the outcome of 603 fresh IVF-ET cycles. In all these cycles there was no male factor infertility and female age was <36 yrs

|  | Male age 20-30 yrs | Male age 31-44 yrs | Male age $\geq$ 40 yrs | P value, Odds ratio and 95%CI |
|---|---|---|---|---|
| Total | 110 (18.2%) | 474 (78.6%) | 19 (3.2%) | |
| Pregnancies | 47 | 179 | 6 | |
| PR /ET | 42.7% | 37.8% | 31.6% | 0.516 (> 0.05) OR and 95% CI 0.761 (0.496 – 1.168) |
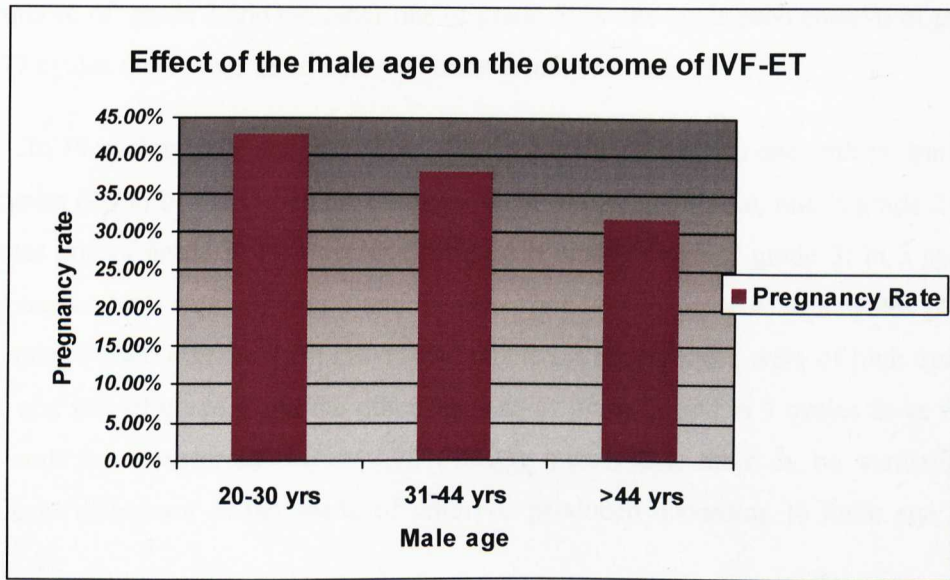
**Figure 2.6** Effect of male age on the outcome of IVF-ET. Differences in the outcome are not statistically significant.

## 2.5.4 Effect of the male age on the grade of embryos:

In our study, there were 110 cycles in which male age was between 20 and 30 years, where there was no male factor infertility and where the female age was less than 36. In 8 cycles there was 1 embryo transfer; in 13(14%) of the cycles the embryos were of low quality one is grade 2 and the other one of grade 3; in 18 cycles (19.4%) each embryo was of grade 3; in 40 cycles (43%) one embryo was grade 3 and the other one was grade 4; in 10 cycles (10.8%) each embryo was grade 4; in 12 cycles (13%) the embryos were of high quality i.e one is grade 4 and the other one is grade 5; and in 9 cycles 3 embryos transferred, see table (2.7).

In 474 cycles, male age was between 31 and 44 years, in 18 of these cycles there was one embryo transfer. In 56 cycles (15%) the embryos were of low quality; in 99 cycles (26.5%) each embryo was grade 3; in 132 cycles (35.3%) one embryo was grade 3 and the other one was grade 4; in 49 cycles (12%) each embryo was of grade 4; in 37 cycles (10%) the embryos were of high quality i.e. one of grade 4 and the other of grade 5; in one cycle one embryo of grade 2 and the other one of grade 5; in 4 cycles

one embryo of grade 3 and the other one of grade 5; in one cycle each embryo of grade 5; in 77 cycles there were three embryos transferred, see table (2.7).

In 19 cycles, male age was above 44. In 2 cycles there was one embryo transfer; in 2 cycles (20%) of the cycles the embryos were of low quality i.e. one is grade 2 and the other one of grade 3; in 2 cycles (20%) each embryo was of grade 3; in 3 cycles (30%) one embryo was of grade 3 and the other one was of grade 4; in 2 cycles (20%) each embryo was of grade 4; in one (10%) of the cycles embryos were of high quality where one was of grade 4 and the other one was of grade 5, and in 7 cycles there were three embryos transferred. P = 0.921(>0.05) means that there is no statistically significant difference in the grade of embryos produced according to male age; see table (2.7).

**Table 2.7** Effect of male age on the grade of embryos. All cycles were fresh IVF cycles and 2 embryos were transferred and female age is <36 years. In this table we can see the proportion of the grades of embryos is equal in all age groups, and there is no statistically significant difference in the distribution of low grade and high grade embryos according to male age.

| Grade of embryos | Male age 20-30 years old 110 cycles | Male age 31-44 years old 474 cycles | Male age >44 years old, 19cycles | Total |
|---|---|---|---|---|
| One embryo grade 2 and one grade 3 | 13 14% | 56 15% | 2 20% | 70 |
| Two embryos both grade 3 | 18 19.4% | 99 26.5% | 2 20% | 129 |
| One embryo grade 3 and one grade 4 | 40 43% | 132 35.3% | 3 30% | 178 |
| Two embryos both grade 4 | 10 10.8% | 49 12% | 2 20 % | 58 |
| One embryo grade 4 and one grade 5 | 12 13% | 37 10% | 1 10% | 47 |
| One embryo grade 2 and one grade 5 | ------- | 1 | ------ | |
| One embryo grade 3 and one grade 5 | --------- | 4 | ------ | |
| Two embryos each grade 5 | -------- | 1 | ------- | |
| Total | 93 | 379 | 10 | 477 |

## 2.5.5 Difference in the outcome between fresh and frozen cycles:

Among 1393 embryo transfer cycles, there were 971 fresh cycles in which 2 embryos were transferred, see table (2.8). 362 cycles produced positive pregnancy tests and the pregnancy and implantation rates were (37.3% and 23.22% respectively). In one cycle there was a transfer of 2 fresh blastocysts and the cycle was positive. In 55 cycles of 2 frozen embryo transfers, 15 cycles were positive and the pregnancy and implantation rates were 27.3% and 16.36% respectively, [P=0.128(P> 0.05), O.R., and 95% CI = 0.626(0.341-1.150)] for the pregnancy rate, and [P = 0.098, Odds ratio, and

95% CI 0.647(0.386-1.084)] for the implantation rate, which means that embryo cryopreservation may not be affecting the outcome but the number of frozen cycles which is available for analysis was small, and this result is contradicting the published analysis, see table (2.9) and figure (2.7). In addition, there were 4 cycles in which embryos were frozen and after thawing they were kept for few days more to reach a blastocyst stage. Of these, 2 cycles were positive and the pregnancy and implantation rates were 50% and 37.5%. In 7 cycles, 1 frozen embryo was transferred, 1 cycle was positive and the pregnancy and implantation rates were 14.3% and 14.3% respectively. In 35 cycles three frozen embryos were transferred, of which 6 cycles were positive and the pregnancy and implantation rates were 17.1% and 5.71% respectively [P (0.460) > 0.05 and the 95% CI = 2.250 (0.250 –20.278)] for the pregnancy rate, and [P 0.046 P<0.05, 95%CI 0.31(0.118-0.814)] for the implantation rate. This means that there is no statistically significant difference in the pregnancy rate according to the number of the embryos used in frozen cycles but there is a statistically significant difference in the implantation rate according to the number of frozen embryos transferred with better outcome with transfer of 2 frozen embryos, see table 2.10(a), and figure (2.8).

**Table 2.8** Number and type of embryos transferred in all IVF-ET, and IVF/ICSI-ET cycles, which are included in the retrospective analysis

| No. of cycles | Type of embryos |
|---|---|
| 247 | 3 Fresh embryos transferred |
| 1 | 3 fresh embryos transferred, 1 of them was morula |
| 1 | 3 fresh embryos transferred, 1 of them was blastocyst |
| 971 | 2 Fresh embryos transferred |
| 1 | 2 fresh blastocyst transferred |
| 71 | 1 Fresh embryo transferred |
| 35 | 3 Frozen embryos transferred |
| 55 | 2 Frozen embryos transferred |
| 7 | 1 Frozen embryo transferred |
| 4 | 2 blastocyst of frozen embryos transferred |
| 1393 | Total |

**Table 2.9.** Effects of embryo cryopreservation on the outcome of IVF and IVF/ICSI-ET cycles. All cycles with transfer of 2 fresh or 2 frozen embryos were included.

| | Fresh cycles of two ET | Frozen cycles of two ET | P (fresh vs. frozen) | OR and 95.0% C.I. | ET of 2 frozen blastocyst |
|---|---|---|---|---|---|
| Total cycles | 971 | 55 | | | 4 |
| Pregnancies | 362 | 15 | | | 2 |
| Pregnancy rate /ET | 37.3% | 27.3% | P= 0.128 (>0.05) | 0.626 (0.341-1.150) | 50% |
| Implantation rate | 23.22% | 16.36% | P = 0.098 (>0.05) | 0.647 (0.386-1.084) | 37.5% |
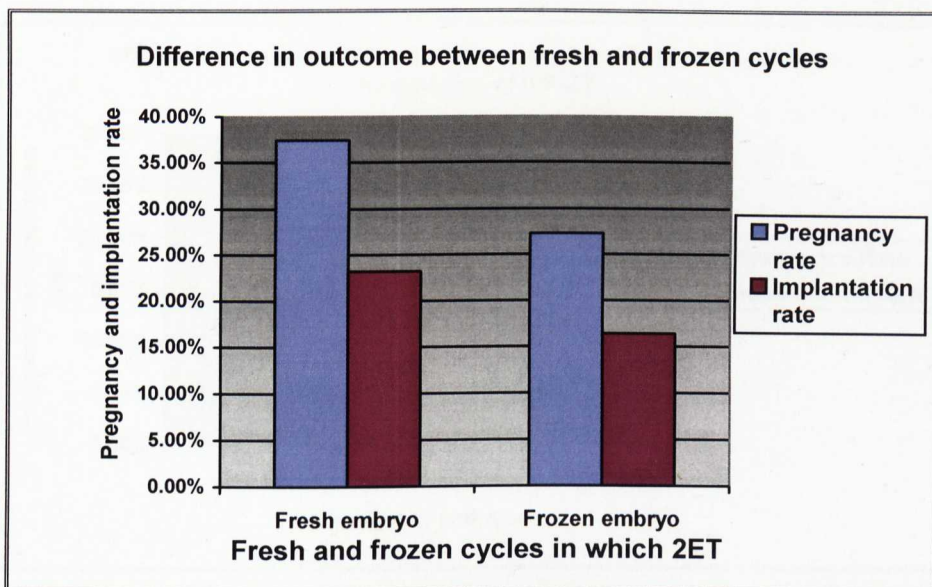


**Figure 2.7** Difference in the outcome between fresh and frozen IVF and IVF/ICSI cycles. The outcome is lower in the frozen cycles, but the differences in pregnancy and implantation rates are not statistically significant

**Table 2.10(a)** Effect of the number of embryos transferred in frozen IVF and IVF/ICSI cycles. Difference was checked with Chi square and Logistic regression tests

|  | total | Frozen one embryo | Frozen 2 embryo | Frozen three embryo | P value | Odds ratio and 95%CI |
|---|---|---|---|---|---|---|
| Total | 97 | 7 | 55 | 35 |  |  |
| Pregnancies | 22 | 1 | 15 | 6 |  |  |
| Pregnancy rate/ ET | 22.68% | 14.3% | 27.3% | 17.1% | 0.460 (> 0.05) | 2.250 (0.250-20.278) |
| Implantation rate | 9.9% | 14.3% | 16.36% | 5.71% | 0.046 (<0.05) | 0.31 (0.118-0.814) |
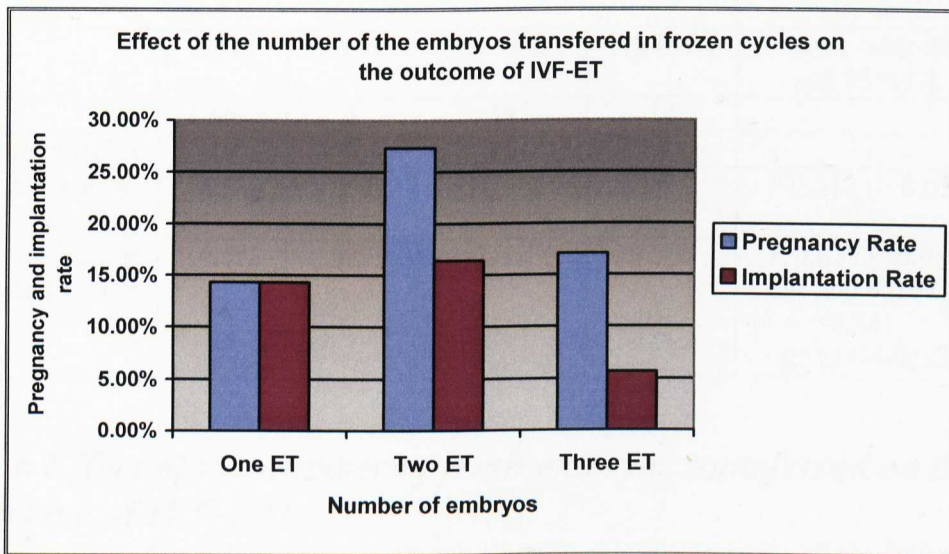


**Figure 2.8** Effect of the number of frozen embryos transferred on the outcome of IVF, and IVF/ICSI cycles. The outcome was good with 2 embryo transfer. Difference in the pregnancy rate is not statistically significant, while difference in the implantation rate of one or two embryo transfers versus three embryo transfer was statistically significant.

When we looked at the cycles in our study, we found that in 42.9% of 1 frozen embryo transfers, 30.9% of the 2 frozen embryo transfers, and in 31.4% of the 3 embryo transfers, female age was above 35 years old. If we include only the cycles in which female age is equal to or below 35 years old, we found that there were 4 cycles in which there was one frozen embryo transfer, all the cycles were negative. In 38 cycles 2 frozen embryos were transferred, 13 cycles were positive and the pregnancy and implantation rates were 34.2% and 21% respectively. In 24 cycles 3 frozen embryos were transferred, 6 cycles were positive and the pregnancy and implantation

rates were 25% and 8.3% respectively. [P (0.312 >0.05)] for the difference in pregnancy rate and [(P 0.035 (<0.05), odds ratio and 95% CI 0.341 (0.125-0.928)] for the difference in the implantation rate. We concluded that even when we exclude all the cycles in which female age was above 35 years old, still the outcome is better with the transfer of two frozen embryos. We could not explain the reason underlying this result, which may be related to a preference to transfer three embryos when they are all of poor quality, see table 2.10(b).

**Table 2.10(b)** Effect of the number of frozen embryos transferred on the outcome of IVF. Only cycles in which female age was less than 36 years old were included.

|  | Total | One ET | Double ET | Triple ET | P value, odds ratio and 95% CI |
|---|---|---|---|---|---|
|  | 66 | 4 | 38 | 24 |  |
| Pregnancies | 19 | 0 | 13 | 6 |  |
| Pregnancy rate/ET | 28.8% | 0% | 34.2% | 25% | P 0.312 (> 0.05) |
| Implantation rate/Embryo | 14.5% | 0% | 21% | 8.3% | P 0.035 (<0.05) OR and 95% CI 0.341 (0.125-0.928) |

## 2.5.6 Effect of the number of fresh embryos transferred on the outcome of IVF-ET:

In our study, there were 71 cycles in which one embryo was transferred, and of these, 12 cycles were positive and the pregnancy and implantation rates were 16.9%. There were 971 cycles in which two embryos were transferred and 362 cycles were positive, making the pregnancy and implantation rates 37.3% and 23.22% respectively. There were 249 cycles in which three embryos were transferred, and in one group of 3 embryos there was one morula, and among another group of 3 fresh embryos there was one fresh blastocyst. 67 cycles were positive and the pregnancy and implantation rates were 27.1% and 11.64% respectively, [P< 0.0001, O.R., and 95% CI 2.935 (1.557–5.534)] for the pregnancy rate, and [P<0.0001, O.R. and 95% CI 1.487(0.792-2.791)] for the implantation rate, which means that there is a statistically significant difference in the outcome according to the number of embryos transferred in fresh cycles see table 2.11(a),(b) and figure (2.9), while in the frozen cycles there was no significant

difference in the pregnancy rate according to the number of embryos transferred, but there was statistically significant difference in the implantation rate according to the number of embryos transferred in frozen cycles, see table 2.10(a) and (b), figure(2.8).

**Table 2.11 (a)** Effect of the number of embryos transferred in fresh IVF and IVF/ICSI cycles. Cycles in which there is a blastocyst and morula were excluded, Difference was checked with Chi square and logistic regression tests

|  | total | One ET | Two ET | Three ET | P | Odds ratio and 95% CI |
|---|---|---|---|---|---|---|
| Total | 1289 | 71 | 971 | 247 |  |  |
| Pregnancies | 441 | 12 | 362 | 67 |  |  |
| Pregnancy rate /ET | 34.29% | 16.9 % | 37.3% | 27.12% | P <0.0001 | 2.935 (1.557-5.534) |
| Implantation rate | 20% | 16.9% | 23.22% | 11.64% | P <0.0001 | 1.487 (0.792-2.791) |
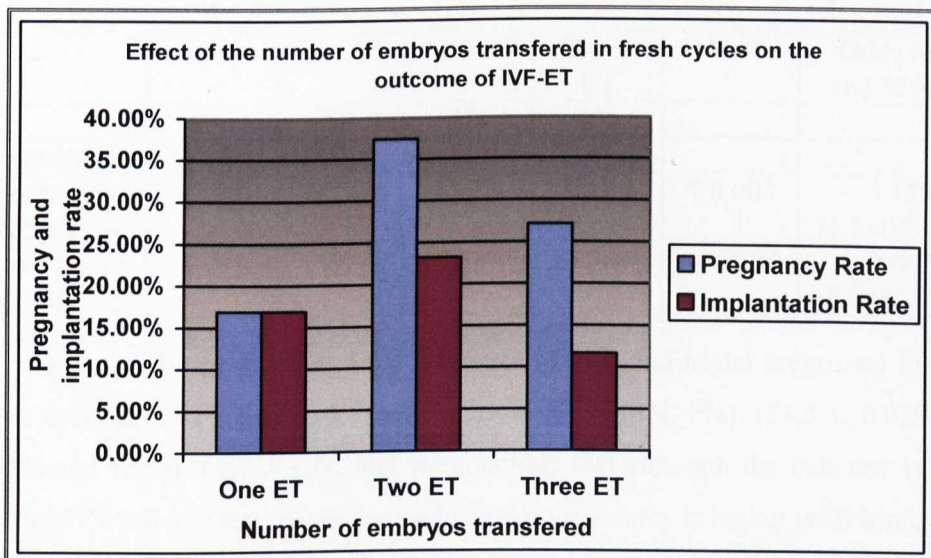


**Figure 2.9** Effect of the number of fresh embryos transferred on the outcome of IVF, and IVF/ICSI cycles. The outcome is good with 2 embryo transfers, and the difference in the pregnancy and implantation rate is statistically significant.

In our study we found that female age was greater than 36 years in (29.6%, 20.6% and 38%) of one, two and three embryo transfers respectively and that if we

exclude these cycles, we found that there were 50 cycles in which there was one embryo transfer, of which 9 cycles were positive, and pregnancy and implantation rates were 18% and 18% respectively. There were 771 cycles in which two fresh embryos were transferred, 314 cycles were positive and pregnancy and implantation rates were 40.7%, 25.68% respectively. In addition, there were 155 cycles of three embryo transfer, 47 cycles were positive and the pregnancy and implantation rates were 30.3%, 13.76% respectively [P<0.001, O.R., and 95% CI 3.130(1.500-6.532)] for the pregnancy rate, and [P<0.0001, O.R. and 95% CI 0.462(0.346-0.616)] for the implantation rate. This means that even after excluding the cycles in which female age was more than 35 years old, the difference in the pregnancy and implantation rates is statistically significant with better outcome from two embryo transfers. We could not explain the underlying cause resulting in the poor outcome of three embryo transfer, which may be related to transfer of poor quality embryos , see table 2.11(b).

**Table 2.11(b)** Effect of the number of embryos transferred in fresh IVF and IVF/ICSI cycles, all the cycles in which the female age is less than 36 years were included. Difference was checked with Chi square and Logistic regression tests

|  | total | One ET | Two ET | Three ET | P Value | Odds ratio and 95% CI |
|---|---|---|---|---|---|---|
|  | 976 | 50 | 771 | 155 |  |  |
| Pregnancies | 370 | 9 | 314 | 47 |  |  |
| Pregnancy rate per ET | 37.9% | 18% | 40.7% | 30.3% | < 0.001 | 3.130 (1.500-6.532) |
| Implantation rate | 22.79% | 18% | 25.68% | 13.76% | <0.0001 | 0.462 (0.346-0.616) |

In our study we found that the incidence of twin and triplet pregnancy in single embryo transfer (SET), double ET, and in triple ET is (0%, 0%), (24.5%, 0.02%) and (26.86% and 1.5%) respectively, and we conclude that although the outcome is lower with triple ET but still the risk of twin and triplet pregnancy is higher with triple ET as compared to double or to single ET, see table 2.11(c), figure (2.10).

**Table 2.11(c)** Proportion of twin and triplet pregnancies in single, double and triple ET

|  | SET | Double ET | Triple ET |
|---|---|---|---|
| twin | 0% | 24.5% | 26.86% |
| triplet | 0% | 0.02% | 1.5% |

**Effect of the number of embryos on the incidence of mutiple pregnancy**
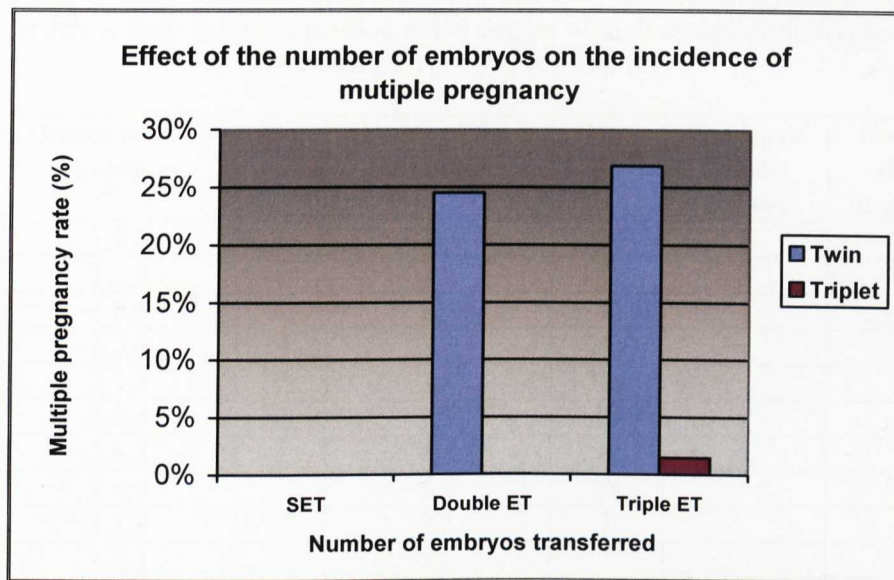
**Figure 2.10** The proportion of twin and triplet pregnancies according to the Number of embryos transferred; the proportion is lowest with 1 ET, and highest with 3 ET.

## 2.5.7 Effect of the grade of embryos on the outcome of IVF-ET:

In table 2.12, we can see the grades of embryos transferred in single, double and triple transfers in 1292 fresh cycles

**Table 2.12** Grade of embryos in all fresh IVF and IVF/ICSI cycles of the retrospective study, embryos were scored according to the degree of their cytoplasmic fragmentation, see figure 2.1(a), (b), (c), (d), (e)

| Grades of the 3 embryos | No. | Grades of the 2 embryos | No. | Grades of one embryo | No. of the cycles |
|---|---|---|---|---|---|
| 1,2,3 | 1 | 2,3 | 165 | 2 | 9 |
| 2,2,2 | 5 | 2,2 | 2 | 3 | 43 |
| 2,2,3 | 30 | 2,4 | 2 | 4 | 19 |
| 2,2,4 | 4 | 3,3 | 247 | | |
| 2,3,3 | 30 | 3,4 | 354 | | |
| 2,3,4 | 18 | 3,5 | 13 | | |
| 2,4,4 | 1 | 4,4 | 99 | | |
| 2,4,5 | 1 | 4,5 | 88 | | |
| 3,3,3 | 37 | 2,5 | 1 | | |
| 3,3,4 | 54 | 5,5 | 1 | | |
| 3,4,4 | 21 | 2 blastocyst | 1 | | |
| 4,4,4 | 10 | | | | |
| 4,4,5 | 13 | | | | |
| 5,5,5 | 2 | | | | |
| 4,4 and one morula | 1 | | | | |
| 3,3,5 | 1 | | | | |
| 3,5,5 | 1 | | | | |
| 2,3 and one blastocyst | 1 | | | | |
| 4,5,5 | 5 | | | | |
| 3,4,5 | 13 | | | | |
| Total    1292 | 249 | | 972 | | 71 |

## 2.5.7.1 Effect of the grade of one embryo on the outcome of IVF-ET:

In our study, there were 9 cycles in which the embryos were of grade 2, and all the cycles were negative. In 43 cycles the embryos were of grade 3, and 7 cycles were positive and the pregnancy rate was therefore 16.3%. In 19 cycles, all the embryos were of grade 4, and 5 cycles were positive with the pregnancy rate being 26.31% P=0.219 (P>0.05) which means that the difference is not statistically significant; see table 2.13 May be with a higher number of cycles the result would reach significance, like in the next subject.

**Table 2.13** Effect of the grade of 1 fresh embryo on the Outcome of IVF, and IVF/ICSI cycles, difference was checked with Chi square test

|  | One embryo grade 2 | One embryo with grade 3 | One embryo with grade 4 | P Value |
|---|---|---|---|---|
| Total | 9 | 43 | 19 |  |
| Pregnancies | 0 | 7 | 5 |  |
| Pregnancy rate/ET | 0% | 16.3% | 26.31 % | 0.219 (>0.05) |

## 2.5.7.2 Effect of the grade of 2 embryos on the outcome of IVF-ET:

Of the 1393 IVF-ET cycles, there were 88 cycles in which one of the embryos was grade 4 and the other one was grade 5. Of these 54 cycles were positive and the pregnancy and implantation rate were (61.4% and 35.8% respectively). In 99 cycles, there were 2 embryos each one of grade 4 of which 51 cycles were positive and the pregnancy and implantation rates were (51.5%, and 33.33% respectively). In 354 cycles one embryo was grade 3 and one was grade 4. Of these 142 cycles were positive and the pregnancy and implantation rates were (40.2%, and 24.92% respectively). In 247 cycles both embryos were grade 3 with 90 cycles positive and the pregnancy and implantation rates being (36.4% and 23.17% respectively). In 165 cycles, one embryo was grade 2 and the other one was grade 3. Of these 19 cycles were positive and the pregnancy and implantation rate was (11.5% and 6.96% respectively) [P< 0.0001, O.R., and 95% C.I. 4.405 (2.557-7.587)] for the pregnancy rate, and [P <0.0001, O.R., and 95% CI 4.004 (2.496-6.424)] for the implantation rate. This means that there is a statistically significant difference in the outcome when there is difference in the grade of the embryos and since the 95% CI ratio is above 1 this means that the higher the grade of the embryo, the better the outcome, see table (2.14), and figure (2.11).

**Table 2.14** Effect of the grade of 2 fresh embryos on the outcome of IVF and IVF/ICSI cycles, only cycles which contain the underlying grades were included, difference was checked with Chi square and logistic regression tests

|  | One embryo grade 4 and one grade 5 | Both embryos grade 4 | One embryo grade 3 and one grade 4 | Both embryos grade 3 | One embryo grade 2 and one grade 3 | P value, Odds ratio and 95% CI |
|---|---|---|---|---|---|---|
| Total | 88 (9.2%) | 99 (10.4%) | 354 (37%) | 247 (26%) | 165 (17.3%) | |
| Pregnancies | 54 | 51 | 142 | 90 | 19 | |
| Pregnancy rate/ET | 61.4% | 51.5% | 40.2% | 36.4% | 11.5% | P < 0.0001 OR and 95% CI 4.405 (2.557 – 7.587) |
| Implantation rate | 35.8% | 33.33% | 24.92% | 23.17% | 6.96% | P <0.0001 OR and 95% CI 4.004 (2.496-6.424) |
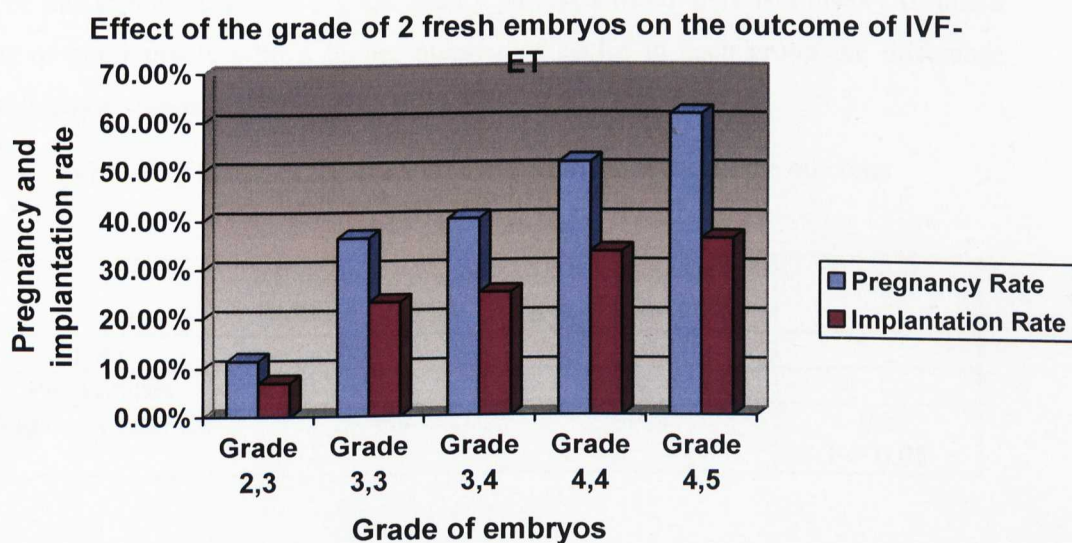


**Figure 2.11** Effect of the grade of 2 fresh embryos on the outcome of IVF and IVF/ICSI cycles. There is stepwise increase in the pregnancy and implantation rates as the grade of embryos becomes higher.

### 2.5.7.3 Effect of the grade of three fresh embryos:

In our study, there were 103 cycles in which 3 embryos were transferred and in each one the embryos were grade 3 or below. In one cycle the 3 embryos were of grade 1, 2, 3, respectively, in 5 cycles each embryo was of grade 2, in 30 cycles the embryos were of grade 2, 2, 3, in 30 cycles the embryos were of grade 2, 3, 3, and in 37 cycles each embryo was of grade 3. Due to the small quantity of data for each group the outcomes of all these groups were collected together for statistical purposes. 20 cycles were positive and the pregnancy rate was 19.4%. In 30 cycles all the embryos were either grade 4 or above. In 10 cycles all the embryos were of grade 4, in 13 cycles the grades of the embryos were 4, 4, and 5, in 5 cycles the embryos were of grade 4, 5, 5 and in 2 cycles the grade of the embryos were 5, 5,5. 9 cycles were positive and the pregnancy rate was 30% P=0.2 (>0.05). This means that there is no statistically significant difference in the outcome between the 2 groups, see table (2.15). This result may be due to the transfer of 3 good quality embryos when there is a history of failed cycles or alternatively with a higher number of cycles in each group the difference might become significant.

**Table 2.15** Effect of the grade of three fresh embryos on the outcome of IVF and IVF/ICSI cycle

|  | All the embryos are grade 3 or below | All the embryos are grade 4 and above | P value |
|---|---|---|---|
| Total | 103 | 30 |  |
| Pregnancies | 20 | 9 |  |
| Pregnancy rate/ ET | 19.4% | 30% | 0.2 P > 0.05 |

## 2.5.8 Effect of the number of blastomeres on the outcome of IVF-ET

In our study, there were 1292 fresh cycles. Of these, 71 cycles were of one embryo transfer, 972 cycles were of 2 fresh embryo transfers, and in 249 cycles there

was the transfer of 3 embryos. In the following 2 tables, table 2.16 (a) and (b) we show the number of the cells in each embryo.

**Table 2.16 (a)** Number of the blastomeres of 1, and 2 fresh embryos transferred in IVF, or IVF/ICSI cycles

| Cells of one embryo | No. of the cycles | Cells of 2 embryos | No. of the cycles | Cells of 2 embryos | No. of the cycles |
|---|---|---|---|---|---|
| 2 cells | 18 | 2,2 cells | 120 | 3,6 | 3 |
| 3 cells | 9 | 2,3 cells | 28 | 3,8 | 1 |
| 4 cells | 21 | 2,4 cells | 99 | 4,4 | 314 |
| 5 cells | 6 | 2 , 5 | 8 | 4,5 | 58 |
| 6 cells | 8 | 2 , 6 | 5 | 4,6 | 51 |
| 8 cells | 9 | 2 , 8 | 3 | 4,8 | 19 |
| | | 3,3 | 9 | 5,5 | 10 |
| | | 3,4 | 48 | 5,6 | 13 |
| | | 3,5 | 10 | 5,8 | 3 |
| | | 8,8 | 71 | 6,6 | 12 |
| | | 6,8 | 86 | 2 fresh blastocyst | 1 |
| total | 71 | | | 972 | |

**Table 2.16 (b)** Number of the blastomeres of 3 embryos transferred in fresh IVF, and IVF/ICSI cycles

| Cells of 3 embryos | No. | Cells of 3 embryos | No. | Cells of 3 embryos | No. |
|---|---|---|---|---|---|
| 2,2,2 | 18 | 3,5,8 | 2 | 5,5,5 | 1 |
| 2,2,3 | 13 | 4,4,4 | 41 | 5,5,8 | 1 |
| 2,2,4 | 13 | 4,4,5 | 10 | 5,8,8 | 2 |
| 2,2,5 | 3 | 4,4,6 | 13 | 6,6,6 | 1 |
| 2,2,6 | 4 | 4,4,8 | 5 | 6,6,8 | 3 |
| 2,2,8 | 3 | 4,5,6 | 4 | 6,8,8 | 13 |
| 2,3,3 | 5 | 4,6,6 | 2 | 2,4,6 | 1 |
| 2,3,4 | 8 | 4,6,8 | 4 | 2,4,8 | 1 |
| 2,3,5 | 1 | 4,8,8 | 3 | 2,5,5 | 1 |
| 2,3,6 | 1 | 4, 8, morula | 1 | 2,5,8 | 1 |
| 2,4,4 | 21 | 2, 3, blastocyst | 1 | 3,3,3 | 2 |
| 2,4,5 | 5 | 8,8,8 | 11 | 3,3,4 | 8 |
| 3,3,5 | 1 | 3,4,4 | 15 | 3,4,6 | 4 |
| 3,4,5 | 1 | | | | |
| | | | | total | 249 |

### 2.5.8.1 Effect of the number of blastomeres of single embryo on the outcome of IVF-ET:

In our study, there were 16 cycles in which there was single embryo transfer on day 2, and the embryos consisted of 2 blastomeres. One cycle was positive and the pregnancy rate was 6.3%. In 18 cycles the embryos consisted of 4 cells and of these, 4 cycles were positive and the pregnancy rate 22.2%, see table 2.17(a). In addition, there were 5 cycles in which the single embryo transfer was done on day 3, of these, 1 cycle was positive and the pregnancy rate was 20%. In 9 cycles the embryos consisted of 8 cells. Of these, 3 cycles were positive and the pregnancy rate was 33.3%; see table 2.17(b).

**Table 2.17(a)** Effect of the number of blastomeres of single embryo transferred on day 2, on the outcome of IVF and IVF/ICSI

|  | One embryo of 2 cells transferred on day 2 | One embryo of 4 cells transferred on day 2 |
|---|---|---|
| Total | 16 | 18 |
| Pregnancies | 1 | 4 |
| Pregnancy rate/ET | 6.3% | 22.2% |

**Table 2.17(b)** Effect of the number of blastomeres of single embryo transferred on day 3, on the outcome of IVF and IVF/ICSI

|  | One embryo of 2 or 3 cell transferred on day 3 | One embryo of 8 cells transferred on day 3 |
|---|---|---|
| total | 5 | 9 |
| pregnancies | 1 | 3 |
| Pregnancy rate/ET | 20% | 33.3% |

### 2.5.8.2 Effect of the number of the blastomeres in two fresh embryos:

Among 1393 IVF-ET cycles, there were 693 cycles in which the 2 fresh embryos were transferred on day 2, and 278 cycles in which the 2 fresh embryos were transferred on day 3 after egg retrieval. For statistical purposes 518 was included in the analysis. In 119 cycles, there were two fresh embryos, each one consisting of 2 cells.

Of these 22 cycles were positive and the pregnancy and implantation rates were 18.3% and 11% respectively. In 97 cycles, there were two fresh embryos consisting of one embryo of 2 cells and the other 4 cells, 33 cycles were positive and the pregnancy and implantation rates were 34%, and 20.2% respectively. There were 302 cycles in which 2 fresh embryos were used with each one consisting of 4 cells. 133 cycles were positive and the pregnancy and implantation rates were 44% and 28% respectively [P<0.0001, O.R., and 95% CI 3.470 (2.072-5.811) for the pregnancy rate, P<0.0001, O.R., and 95% CI 3.138 (2.012-4.894)] for the implantation rate; see table 2.18(a) and figure (2.12).

From 278 cycles in which two fresh embryos were transferred on day 3, 171cycles were included in the analysis for statistical purposes. In 15 cycles, the embryos consisted of only a few blastomeres, two of 2 cells in one cycle, one of 2 and one of 4 cells another cycle and two of 4 cells in the remaining 13 cycles. Of these 15 cycles, 3 were positive and the pregnancy and the implantation rates were 20% and 13.3% respectively. In 86 cycles, 2 embryos were obtained with one embryo consisting of 6 cells and the other one consisting of 8 cells. Of these, 45 cycles were positive and the pregnancy and the implantation rates were 52.3% and 34.11% respectively. In 71 cycles each embryo consisted of 8 cells, 45 cycles were positive and the pregnancy and implantation rates were 63.4% and 40.14% respectively [P <0.017, O.R. 4.5 and 95% CI = (1.184 – 17.099)] for the pregnancy rate, and [P <0.027, O.R. 3.279 and 95% CI (1.092-9.848)] for the implantation rate. This means that there is a statistically significant difference in the outcome depending on the number of cells for the same developmental age, with better outcomes with higher numbers of cells, see table 2.18(b) and figure (2.13). We concluded that, the outcome is better with higher number of blastomeres for the same gestational age.

**Table 2.18(a)** Effect of the number of the blastomeres of 2 fresh embryos transferred on day 2, on the outcome of IVF and IVF/ICSI-ET cycles, the number in between the bracket indicate the number of the blastomeres of each embryo, all the cycles contain these number of blastomeres were included. The difference was checked with Chi square, and logistic regression test.

|  | (2,2) cells | (2,4) cells | (4,4) cells | P value | Odds ratios and 95% CI |
|---|---|---|---|---|---|
| Total | 119 | 97 | 302 |  |  |
| pregnancies | 22 | 33 | 133 |  |  |
| Pregnancy rate/ET | 18.3% | 34% | 44% | <0.0001 | 3.470 (2.072-5.811) |
| Implantation rate | 11% | 20.2% | 28% | <0.0001 | 3.138 (2.012 - 4.894) |
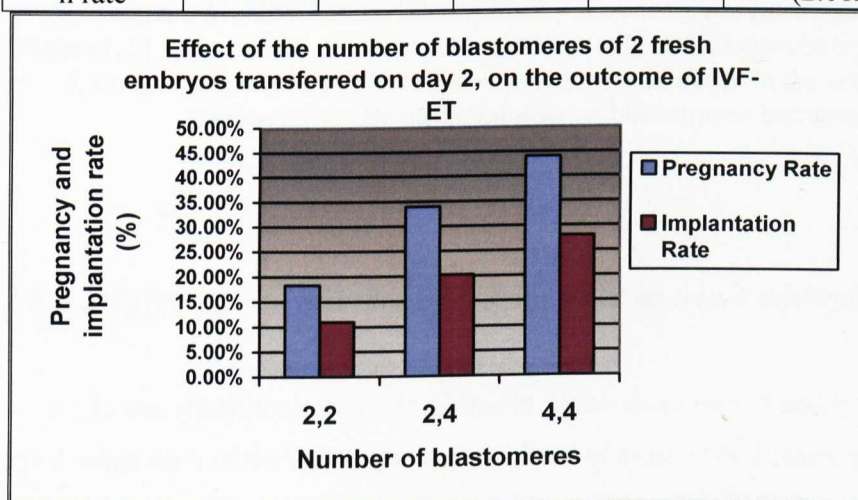


**Figure 2.12** Effect of the number of blastomeres of 2 fresh embryos transferred on day 2 on the outcome of IVF-ET. There is a stepwise increase in the Pregnancy and implantation rate as the number of blastomeres increases.

**Table 2.18(b)** Effect of the number of blastomeres of 2 fresh embryos transferred on day 3, on the outcome of IVF-ET. The numbers in brackets indicates the number of blastomeres of each embryo.

|  | (4,4) | (6,8) | (8,8) | P value | Odds ratio, 95% CI |
|---|---|---|---|---|---|
| Total | 15 | 86 | 71 |  |  |
| Positive | 3 | 45 | 45 |  |  |
| Pregnancy rate | 20% | 52.3% | 63.4% | 0.017 | 4.5 (1.184-17.099) |
| Implantation rate | 13.3% | 34.11% | 40.14% | 0.027 | 3.279 (1.092-9.848) |
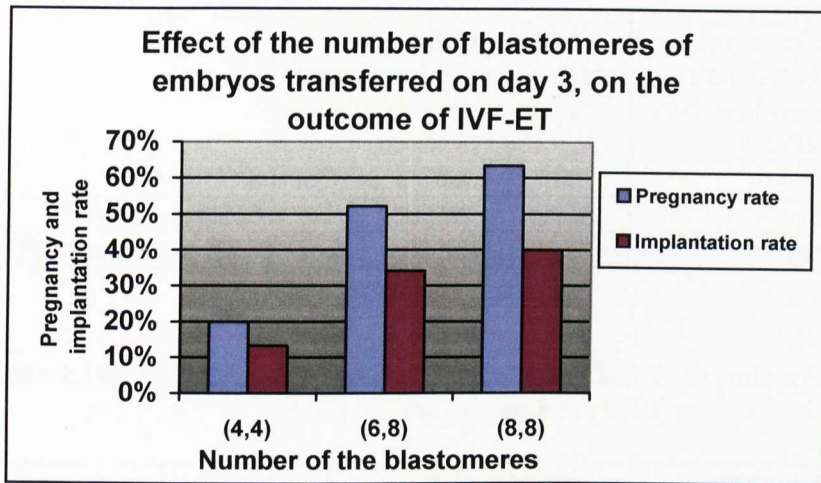
**Figure2.13** Effect of the number of blastomeres of 2 fresh embryos transferred on day 3, on the outcome of IVF-ET. There is stepwise increase in the pregnancy and implantation rate as the number of blastomeres increases.

### 2.5.8.3 Effect of the number of blastomeres in triple embryo transfers:

In our study, there were 34 cycles in which there were 3 embryos transferred on day 2 when each of the three embryos consisted of either 2 or 3 blastomeres. Of these, 9 cycles were positive and the pregnancy rate was therefore 26.47%. In addition, there were 44 cycles in which each of the 3 embryos consisted of either 4 or 5 cells. Of these 44 cycles, 15 cycles were positive, and the pregnancy rate was therefore 34%, see table 2.19(a). There were 10 cycles in which the 3 embryos transferred on day 3 each consisted of 4 cells or less, and of these, only 2 cycles were positive, and the pregnancy rate was therefore 20%. There were 23 cycles of day 3 embryo transfer when at least 2 embryos consisted of 8 cells (in 13 cycles 6,8 and 8 cells, and in 10 cycles all embryos had 8 cells). Of these 23 cycles, 11 were positive and the pregnancy rate was therefore 47.82%; see table 2.19(b).

**Table 2.19 (a)** Effect of the number of blastomeres in 3 fresh embryos transferred on day 2 on the outcome of IVF-ET

|  | Cycles of 3 embryos transferred on day 2, each embryo consisting of 3 cells or less | Cycles of 3 embryos transferred on day 3, each embryo consisting of 4 cells more |
|---|---|---|
| Total | 34 | 44 |
| Pregnancies | 9 | 15 |
| Pregnancy rate/ET | 26.47% | 34 % |

**Table 2.19(b)** Effect of the number of blastomeres in 3 fresh embryos transferred on day 3, on the outcome of IVF-ET

|  | Cycles of 3 embryos transferred on day 3, each embryo consisting of 4 cells or less | Cycles of 3 embryos transferred on day 3, each embryo consisting of 6 cells or more |
|---|---|---|
| Total | 10 | 23 |
| Pregnancies | 2 | 11 |
| Pregnancy rate/ET | 20% | 47.82% |

## 2.5.9 Day 2 versus day 3 embryo transfer:

In our study, there were 693 cycles (71.4%) in which 2 fresh embryos were transferred on day two. Of these 243 cycles were positive and the pregnancy and implantation rates were (35.1% and 21.53% respectively). In 278 cycles (28.6%) in which 2 fresh embryos were transferred on day three, 119 cycles were positive and the pregnancy and implantation rates were (42.8% and 27.43% respectively), [P = 0.024 (< 0.05 ), and 95% CI = 1.024 (1.043-1.842)] for the pregnancy rate, and [P<0.005, Odds ratio 1.378, and 95% CI(1.099-1.728)] for the implantation rate. This means that there is a statistically significant difference in the outcome between ET on day 2 when compared with transfer on day 3, with significantly better outcomes when the transfer done on day three, this result might be biased due to embryo transfer was done according to the day of egg collection, see table (2.20), and figure (2.14).

**Table 2.20** Day 2 versus day 3 embryo transfer. All cycles with 2 fresh embryos transferred were included, the difference was checked with Chi square and logistic regression tests

|  | 2 embryo transfer | Day 2 embryo transfer (71.4%) | Day 3 embryo transfer (28.6%) | P Value | Odds ratio and 95% CI |
|---|---|---|---|---|---|
| Total | 971 | 693 | 278 |  |  |
| Pregnancies | 362 | 243 | 119 |  |  |
| Pregnancy rate/ET | 37.3% | 35.1% | 42.8% | P<0.024 | 1.024 (1.043-1.842) |
| Implantation rate/ET | 23.22% | 21.53% | 27.43% | <0.005 | 1.378 (1.099-1.728) |
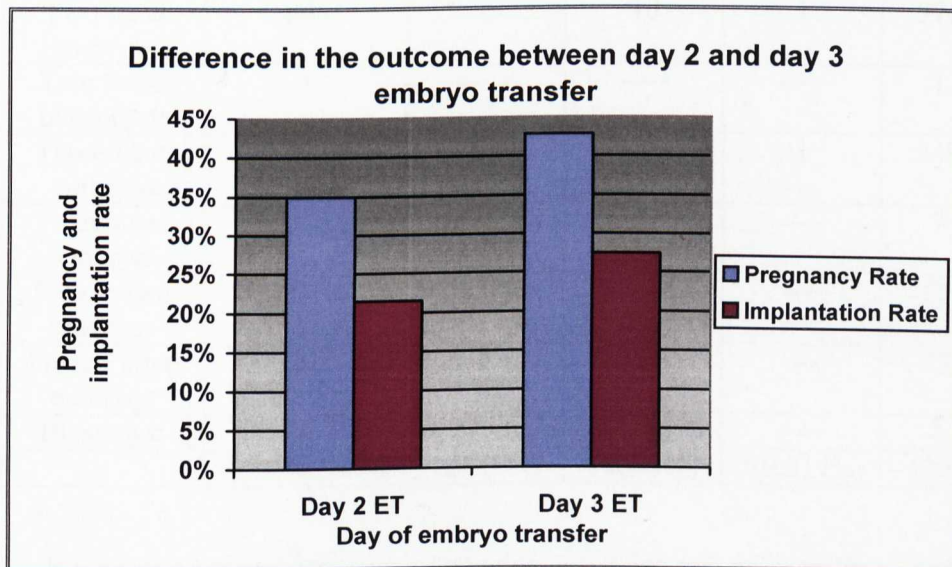


**Figure 2.14** Day 2 versus day 3 embryo transfer. This figure shows that there is a significantly better outcome of day 3 embryo transfers as compared to day 2 embryo transfer.

## 2.5.10 Effect of the procedure, whether TCET (easy or difficult), or TMET:

In our study, we found that, there were 1375 (98.8%) cycles in which the procedure used was TCET (Trans cervical embryo transfer). In 124 (9%) cycles the procedures were difficult and the remaining 1251 (89.8%) were easy. In just 17 cycles (1.2%) the procedure of embryo transfer was TMET (Trans myometrial embryo transfer), in one of the 17cycles the patient was an egg sharing, and 2 cycles were ZIFT (Zygote intrafallopian embryo transfer), see table 2.21(a).

**Table 2.21(a)** Distribution of the TCET (easy or difficult) or TMET according to the type of embryos transferred

| Type of the embryo | TCET easy | TCET difficult | TMET | ZIFT | Total |
|---|---|---|---|---|---|
| One fresh embryo | 67 | 4 | ----- | ----- | 71 |
| Two fresh embryos | 865 | 95 | 10 | 1 | 971 |
| Two Fresh blastocysts | 1 | ------- | ----- | ----- | 1 |
| Three fresh embryos | 222 | 22 | 4 | 1 | 249 |
| Frozen one embryo | 6 | --- | 1 | ----- | 7 |
| Frozen two embryos | 54 | 1 | --- | ---- | 55 |
| Frozen three embryos | 32 | 2 | 1 | ---- | 35 |
| Blastocyst | 4 | --- | --- | ----- | 4 |
|  | 1251(89.8%) | 124 (9%) | 16(1.2%) | 2(0.014%) | 1393 |

If we select just the outcomes of transfers involving two fresh embryos from our data we find that there were 95 cycles in which the transfer of two fresh embryos was transcervical and difficult. These procedures were time consuming as the catheter was changed, the embryos were reloaded and a tenaculum was used. Of these, 38 cycles were positive and the pregnancy and implantation rates were 40% and 26.31% respectively. In addition, there were 865 cycles in which the transfer of two fresh embryos was transcervical but they were easy and smooth. Of these, 323 cycles were

positive and the pregnancy and implantation rates were 37.3% and 23.1% respectively. Furthermore, there was a total of 16 cycles in which the transfer of the embryos was transmyometrial and of these, there were 10 cycles that involved the transfer of 2 fresh embryos while in 4 cycles there was the transfer of 3 fresh embryos and in one cycle there was the transfer of one frozen embryo. In one other cycle, there was the transfer of three frozen embryos. The indications for TMET were either previous impossible embryo transfer or very difficult mock embryo transfer due to cervical stenosis. The other indication was the presence of a false passage in the cervical canal. In all the cases the TMET was easy. The cycle was only positive in one case and the pregnancy and implantation rates were 10%, and 10% respectively if we chose only 2 fresh embryo transfer and (6.25%, 5.5% respectively) if we chose all the TMET, [P = 0.612 (>0.05), and odds ratio and 95% CI was 1.119 (0.726- 1.725)]. This means that there is no statistically significant difference in the outcome between easy and difficult TCET and that the outcome of the TMET is lower, with better outcomes following TCET, even if it is difficult, see table 2.21(b), and figure (2.15).

**Table 2.21(b)** Difference in the outcome between (TCET) easy or difficult and (TMET). All IVF, and IVF/ICSI cycles with 2 fresh embryo transfers were included. Difference was checked with Chi square, and Logistic regression tests

|  | Total | TCET ( easy ) | TCET ( Difficult ) | P Value, Odds ratio and 95% CI | TMET |
|---|---|---|---|---|---|
| Total | 970 | 865 | 95 |  | 10 |
| Pregnancies | 362 | 323 | 38 |  | 1 |
| Pregnancy rate/ET | 37.31% | 37.3% | 40% | 0.612  P>0.05 95% CI 1.119(0.726-1.725) | 10% |
| Implantation rate | 23.2% | 23.1% | 26.31% | 0.242 >0.05 95% CI 1.192(0.847-1.677) | 10% |

**Difference in the outcome between TCET(easy or difficult)and TMET**
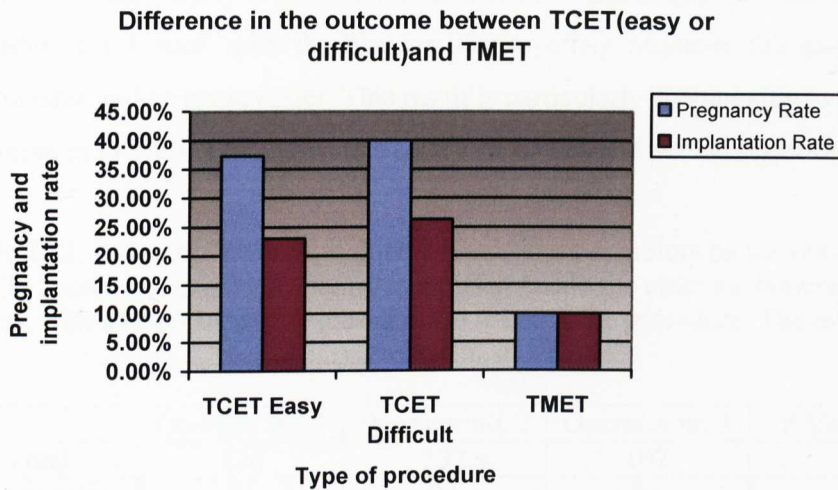


**Figure 2.15** Effect of the procedure TCET (easy, difficult), or TMET on the outcome of IVF-ET. The outcome is better with TCET. There is no statistically significant difference in the outcome of TCET easy or difficult, and the outcome of TMET is low

## 2.5.11 Effect of different operators on the outcome of IVF- ET:

Our data shows that 1393 ET procedures were done by 7 different operators, and that the ET procedures were mainly done by new trainees or by a nurse. For our study we compared the outcomes of 575 ET procedures performed by 3 different operators in cycles when a good prognosis was expected. All the patients were less than 36 years old, with the transfer of two embryos of at least grade 3, all of them had easy mock embryo transfer, and all the ETs were easy, smooth, no tenaculum was used, and no catheter was changed. The first operator did 236 ET procedures of which 83 procedures were positive and the pregnancy rate was 35.2%. The second operator did 237 ET procedures of which 99 were positive and the pregnancy rate was 41.7%. The third operator did 102 ET procedures with 27 positive and the pregnancy rate being 26.5%. [P = 0.026 (P < 0.05)], see table (2.22) and figure (2.16). This means that there is a statistically significant difference in the outcome between the three operators. It is an important point to note that operators' number 1 and number 3 are doctors and they were new trainees while operator number 2 is a nurse. It is also important that all the procedures selected were easy and that no tenaculum was used. The nurse is used to managing many difficult procedures in Hull IVF unit. Sometimes she will attempt a

procedure and if she feels it is difficult due to utero-cervical angulation, she may delay the procedure for 1 hour, until the bladder of the patient becomes full and thus the embryo transfer will be made easier. This result is particularly encouraging to assess the role of nurses in IVF-ET with these satisfactory outcomes and no complications.

**Table 2.22.** Effects of procedure done by 3 different operators on the outcome of IVF-ET. There is a statistically significant difference in the outcome between the 3 operators, with a better outcome when a nurse is doing the procedure. The difference was checked with Chi square test.

|  | Operator no. 1 | Operator no. 2 | Operator no.3 | P Value |
|---|---|---|---|---|
| Total | 236 | 237 | 102 | |
| Pregnancies | 83 | 99 | 27 | |
| Pregnancy rate / ET | 35.2% | 41.7% | 26.5% | < 0.026 |
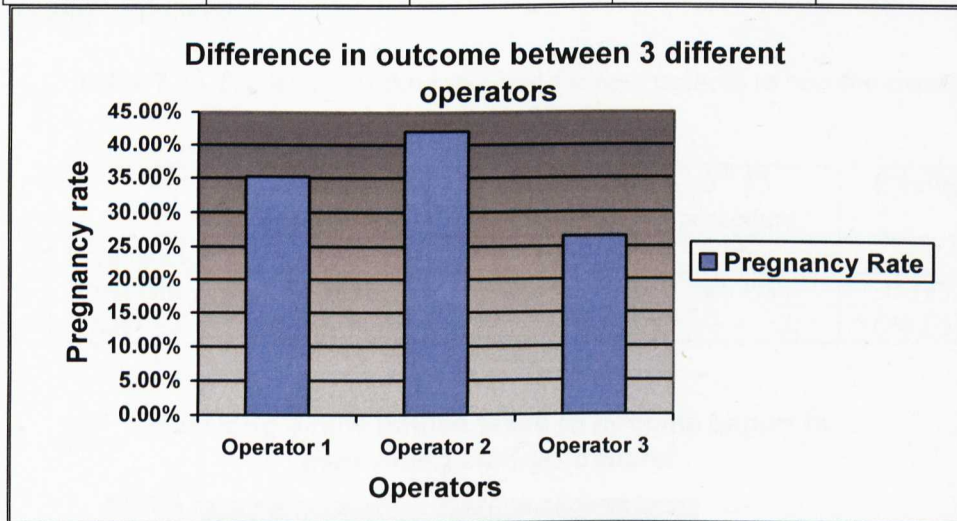


**Figure 2.16** Effect of the procedure done by 3 different operators on the outcome of IVF-ET. All the procedures were easy, smooth, and no tenaculum were used and operator no.2 is a nurse while operators nos.1 and 3 were new trainee doctors.

## 2.5.12 How long does a new trainee take to become expert in embryo transfer?

The nurse who is doing the embryo transfer underwent a training programme following which she was eventually assessed by a senior doctor and was certified

competent in performing embryo transfer. When difficulties arose with the procedure,
such as having to apply a vulsellum forceps to the cervix or to perform a cervical
dilatation, a doctor was called in to perform the procedure.

The outcomes of embryo transfer procedures done by the nurse while she was a
new trainee were analysed. In the first 55 procedures, 19 out of 55 procedures was
positive and the success rate was 34.54 %. In the second 55 procedures, 22 out of 55
procedures were positive and the success rate was 40%. In the third 55 procedures, 26
out of 55 procedures was positive and the success rate was 47.27%. [P = 0.395 (P >
0.05)], see table (2.23), and figure (2.17). This high percentage of success is
encouraging and may suggest leaving all the straightforward embryo transfer operations
to be done by the nurse and to encourage the remaining nurses to enter the same
programme of training.

**Table 2.23** The length of time required for new trainees to become expert in
doing embryo transfer

|  | First 55 procedures | 2nd 55 procedure | 3rd 55 procedure | P value |
|---|---|---|---|---|
| Pregnancies | 19 | 22 | 26 | |
| Pregnancy rate/ ET | 34.54% | 40% | 47.3% | 0.395 (>0.05) |
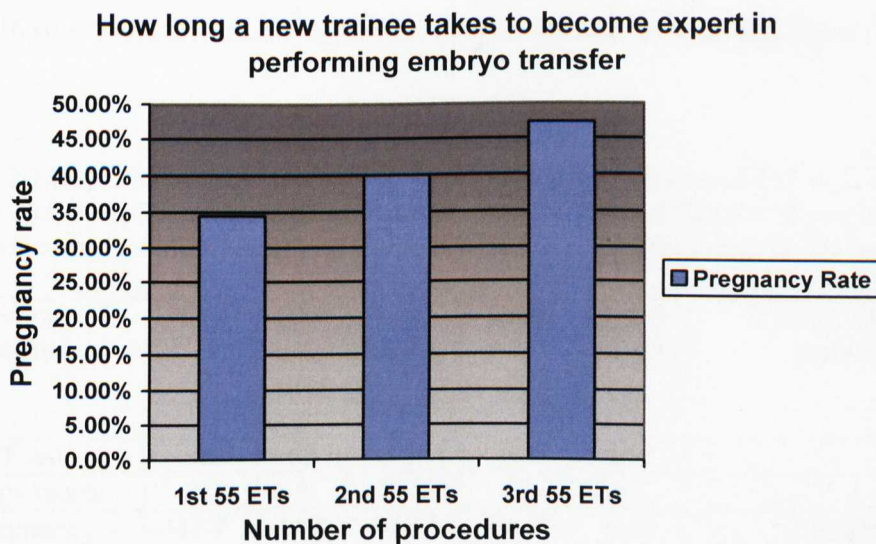


**Figure 2.17** How long does a new trainee will take to become expert in doing embryo
transfer? New trainees should carry out at least 100 embryo transfer procedures before
they become expert in doing embryo transfer procedures.

## 2.5.13 Effect of the causes of infertility on the outcome of IVF-ET:

In our study, 715 cycles were selected. In all these cycles only pure causes were included and in all these cycles either two or three embryos were transferred, or the female age was less than 36 years. There were 48 cycles in which the cause of infertility was PCOS and 20 of these cycles were positive and the pregnancy and implantation rate 41.7%, and 27.52% respectively. There were 68 cycles in which the cause of infertility was endometriosis, 8 of these cycles were positive and the pregnancy and implantation rates were 11.8%, and 7.58% respectively. There were 344 cycles in which there was male factor infertility, 132 of these cycles were positive and the pregnancy and implantation rates were 38.37%, and 21.85% respectively. There were 105 cycles in which the cause of infertility was pure tubal, "no hydrosalpinx and no severe pelvic adhesions" 65 of these cycles were positive and the pregnancy and implantation rates were 61.9%, and 37.55% respectively. There were 140 cycles in which the cause of infertility was unexplained and 65 of these cycles were positive and the pregnancy and implantation rates were 46.4%, and 29.62% respectively. In 10 cycles with severe pelvic adhesion all were negative. [$P < 0.0001$, Odds ratio, and 95% CI = 0.187 (0.73-0.475)] for the pregnancy rate, and [$P < 0.0001$, Odds ratio, and 95% CI 0.216 (0.103-0.455)] for the implantation rate, see table 2.24(a), and figure (2.18).

**Table 2.24(a)** Effect of the aetiology of infertility on the outcome of IVF-ET. All fresh IVF, and IVF/ICSI cycles with pure cause of infertility, and transfer of two or three embryos were included, result was checked with logistic regression and Chi square test

| Aetiology of infertility | PCOS | Endometriosis | Male factor | Tubal | Unexplained | P value, Odds ratio, and 95% CI |
|---|---|---|---|---|---|---|
| Total | 48 | 68 | 344 | 105 | 140 | |
| Pregnancies | 20 | 8 | 132 | 65 | 65 | |
| Pregnancy Rate/ET | 41.7% | 11.8% | 38.37% | 61.9% | 46.4% | P<0.0001 95% CI 0.187 (0.73 -0.475) |
| Implantation rate | 27.52% | 7.58% | 21.85% | 37.55% | 29.62% | P < 0.0001, 95% CI 0.216 (0.103-0.455) |
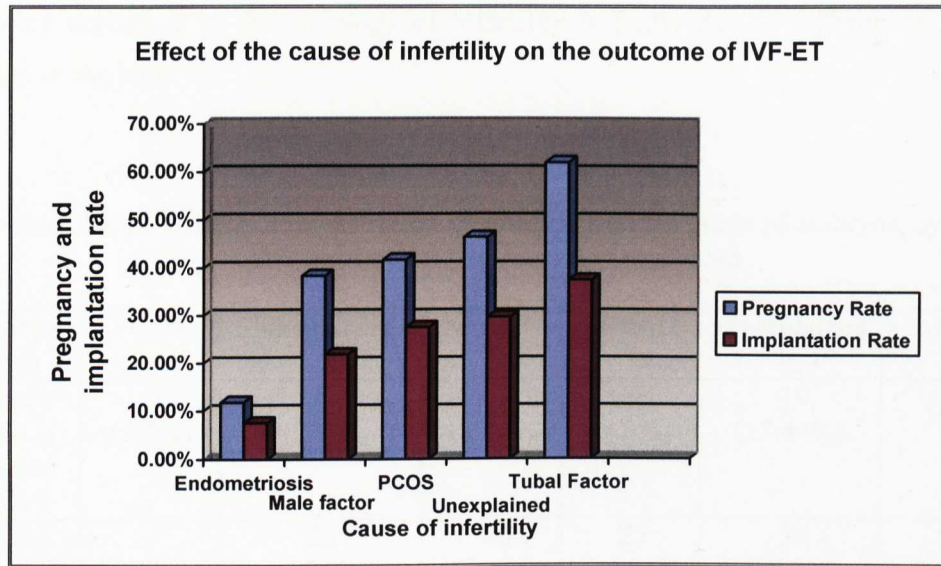
**Figure 2.18.** Effect of the aetiology of infertility on the outcome of IVF, and IVF/ICSI ET. The outcome is highest when the aetiology of infertility is tubal, PCOS or unexplained.

## 2.5.13.1 Effect of the cause of infertility on the grade of embryo:

In our study, we compared the relationship between the grade of embryos and the cause of infertility, in all cycles, female age is less than 36 years so that there will be no effect of the female age on the grade of the embryo. We found that the highest proportion of low grade embryos was amongst the cycles in which there was endometriosis, "22% of the 2 fresh embryos were of grade 2,3 and 35% of grade 3,3" which means that in around 57% of the cycles there was no embryos of grade 4, while in the cycles in which the aetiology of infertility were PCOS, Male factor, tubal and unexplained, there were in 26%, 43%, 34% and 35% of the cycles respectively no embryos of grade 4. We also found that the highest percentage of high grade embryos was amongst the cycles in which there was PCOS "20% of grade (4, 5). In addition, there was one cycle in which 2 embryos each one was grade 5, and 2 cycles in which the 2 embryos one is grade 3, and one is grade 5, see table 2.24(b), and figure (2.19). The difference was checked by Chi square test. P = 0.051 which means that the difference in the distribution of the grades of the embryos according to the cause of

infertility is just statistically significant. This probably explains the difference in the outcome according to the aetiology of infertility is partly due to differences in the quality of the embryos.

**Table 2.24(b).** Effect of the aetiology of infertility on the grade of embryos, cycles with 2 fresh embryo transfer were included

| Embryo grade | PCOS (35) | Endometriosis 59 | Male F. 266 | Tubal 94 | Unexplained 123 | P |
|---|---|---|---|---|---|---|
| One grade 2, and one grade 3 | 2 (5.7%) | 13 (22%) | 43 (16.16%) | 9 (9.6%) | 19 (15.44%) | |
| Two embryos each one grade 3 | 7 (20%) | 21 (35.5%) | 72 (27%) | 23 (24.5%) | 24 (19.5%) | |
| One grade 3 and one grade 4 | 14 (40%) | 17 (28.8%) | 100 (37.6%) | 42 (44.7%) | 44 (35.7%) | |
| Two embryos each one grade 4 | 2 (5.7%) | 5 (8.4%) | 22 (8.27%) | 13 (13.8%) | 18 (14.6%) | |
| One grade 4 and one grade 5 | 7 (20%) | 3 (5%) | 26 (9.77%) | 7 (7.4%) | 17 (13.8%) | 0.051 |
| One grade 3, and one grade 5 | 2 | ------ | 3 | ------ | one | |
| Two embryos each one is grade 5 | one | ------- | ------- | ----- | ------ | |
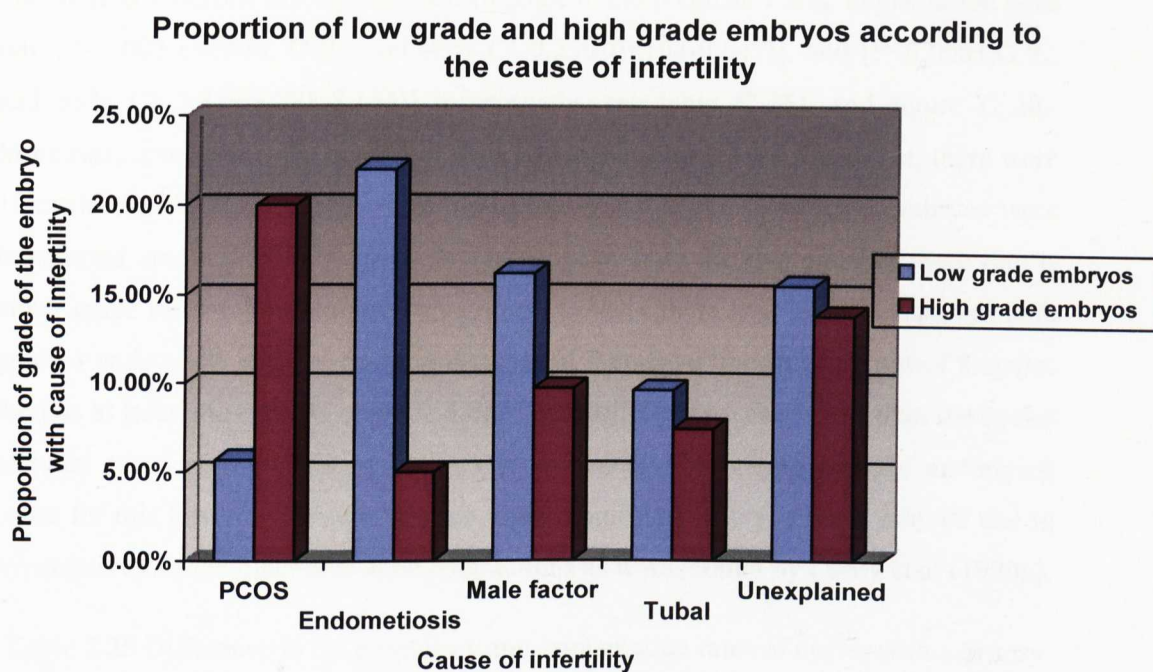
**Figure 2.19** Effect of aetiology of infertility on the grade of embryos. This figure shows that the highest proportion of low grade embryos were among the cycles in which there was endometriosis, and the lowest proportion of low grade embryos were among the cycles in which there was PCOS, while the proportion of high grade embryos were highest among the cycles in which there was PCOS, and the lowest proportion of the high grade embryos were among the cycles in which there was endometriosis

## 2.5.14 The outcome of IVF-ET in patients with a history of ectopic pregnancy:

In our study, there were 47 cycles in which the patients had a history of ectopic pregnancy, in 21 cycles the ectopic was bilateral, and the remaining was unilateral ectopic pregnancy, 10 cycles were positive and the pregnancy rate was 21.3%, and if we chose only the cycles in which there is 2 or 3 fresh embryo transfer and the female age is equal or less than 35 years, we found that there is 27 cycles, 8 of them are successful and the pregnancy and the implantation rate were 29.6% and 14.5% respectively, and if we compare this outcome with the cycles of tubal cause, the pregnancy and the implantation rates were 61.9% and 37.55% respectively, we found

that there is a statistically significant difference in the pregnancy and implantation rates rate [P=0.003 (<0.05), O.R., and 95% CI 0.259 (0.104-0.647)], and [P<0.0001,O.R., and 95% CI 3.819(1.788-8.154)] respectively, see table (2.25), and figure (2.20). Moreover, if we check the quality of embryos in these cycles we found that, there were 19 cycles in which 2 embryos were transferred and 8 cycles in which 3 embryos were transferred, and a part from 19 cycles, in 5 cycles only the two embryos each one is either grade two or three, and the remaining 14 cycles there is at least one embryo with grade 4 or 5. while in the remaining 8 cycles of 3 embryo transfer, in 6 out of 8 cycles there is at least one embryo of grade 4 or 5, and still the outcome is less than the cycles of tubal cause with out history of ectopic pregnancy, this means that the underlying cause for this lower outcome is not due to the quality of embryos but it may be due to hyperactivity of the junctional zone contractions as it was found by Lesny et al (1999b).

**Table 2.25** Difference in the pregnancy and implantation rates of cycles with a history of ectopic pregnancy as compared with the cycles of tubal cause of infertility. Only cycles with 2 or 3 embryos transferred and female age less than 36 years were included.

|  | Patients with H/O ectopic pregnancy | Patients with tubal cause of infertility | P value, Odds ratio, and 95% CI |
|---|---|---|---|
| Pregnancy rate /ET | 29.6% | 61.9% | P<0.003 Odds ratio and 95% CI 0.259 (0.104-0.647) |
| Implantation rate/Embryo | 14.5% | 37.55% | P<0.0001 Odds ratio and 95% CI 3.819 (1.788-8.154) |
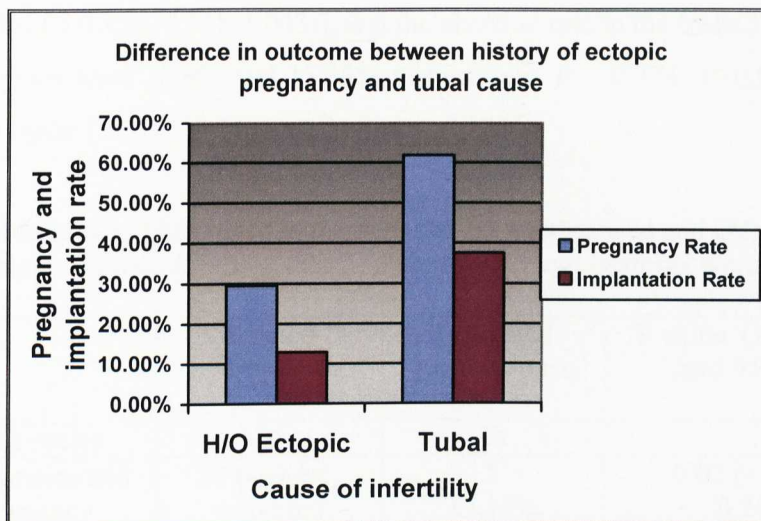
**Figure2.20** Difference in the pregnancy and implantation rate between cycles in which patients had a history of ectopic pregnancy, and other cycles with tubal cause. This figure shows that the outcome is lower with a history of ectopic as compared with other cases of tubal infertility

## 2.5.15 The outcome for patients with hydrosalpinx:

We had 64 cycles in which the patients had hydrosalpinx. In 26 of these cycles the patients had a bilateral salpingectomy, 10 cycles were positive and the pregnancy rate was 38.46%. 4 cycles ended with miscarriages, the implantation, abortion and the live birth rates were 18%, 40% and 23% respectively. In 38 cycles, the hydrosalpinx were untreated and in two of these, there was intrauterine fluid accumulation, both these cycles were negative. Out of 38 cycles, 5 cycles were positive and the pregnancy, implantation, and live birth rates were 13.15%, 6%, and 10.52% respectively, see table 2.26(a). If we chose only the cycles with female age less than 36 years and had two or three embryo transfer, the pregnancy and implantation rates for the treated Hydrosalpinges and the untreated hydrosalpinx cycles were 36.84% versus 14.3% respectively, and 18.18% versus 6.36% respectively. If we compare the outcome of the hydrosalpinx cycles with the outcome of tubal factor in which the pregnancy, implantation, and live birth rates were 61.9%, 37.55%, and 50.5% respectively, we find that there is a statistically significant difference in the pregnancy, implantation and live birth rates between the three groups of patients [P<0.001,O.R., and 95% CI =9.512 (2.636-34.323)],[P < 0.0001 , O.R., and 95% CI 0.113 (0.034-0.377)], and [P < 0.006,

O.R. and 95% CI 0.350 (0.118-1.043)], and the abortion rate in the treated hydrosalpinx and tubal cycles were 28.6% and 15.38% respectively, P = 0.374 (P>0.05), see table 2.26(b) and figure (2.21).

**Table 2.26(a)** Difference in the outcome between treated and untreated hydrosalpinx cycles. All the cycles of patients with hydrosalpinx were included

| | Treated hydrosalpinx | Untreated hydrosalpinx | P value, Odds ratio and 95% CI |
|---|---|---|---|
| Total cycles | 26 | 38 | |
| Pregnancies and Pregnancy rate/ET | 10 positive, 4 aborted 38.46% | 5 13.15% | 0.02 (< 0.05) 0.242 (0.071-0.828) |
| Implantation rate/Embryo | 11/61 18% | 5/83 6 % | 0.03(<0.05 ) 0.291(0.096-0.889) |
| Live birth rate/ET | 23% | 10.52% | 0.3 (> 0.05) |

**Table 2.26(b)** Difference in the outcome of untreated hydrosalpinx, treated hydrosalpinx and tubal cause of infertility. Only cycles with transfer of two or three fresh embryos and with female age less than 36 years were included

| | Un treated hydrosalpinx | Treated hydrosalpinx | Tubal factor | P value, odds ratio and 95% CI |
|---|---|---|---|---|
| Pregnancy rate/ET | 14.3% | 36.84% | 61.9% | P < 0.001, 95% CI 9.512(2.636-34.323) |
| Implantation rate/Embryo | 6.36% | 18.18% | 37.55% | P < 0.0001, 95% CI 0.113 (0.034-0.377) |
| Live birth rate | 14.3% | 26.3% | 50.5% | P < 0.006, 95% CI 0.350 (0.118-1.043) |
| Abortion rate | ----- | 28.6% | 15.38% | 0.374 > 0.05 |
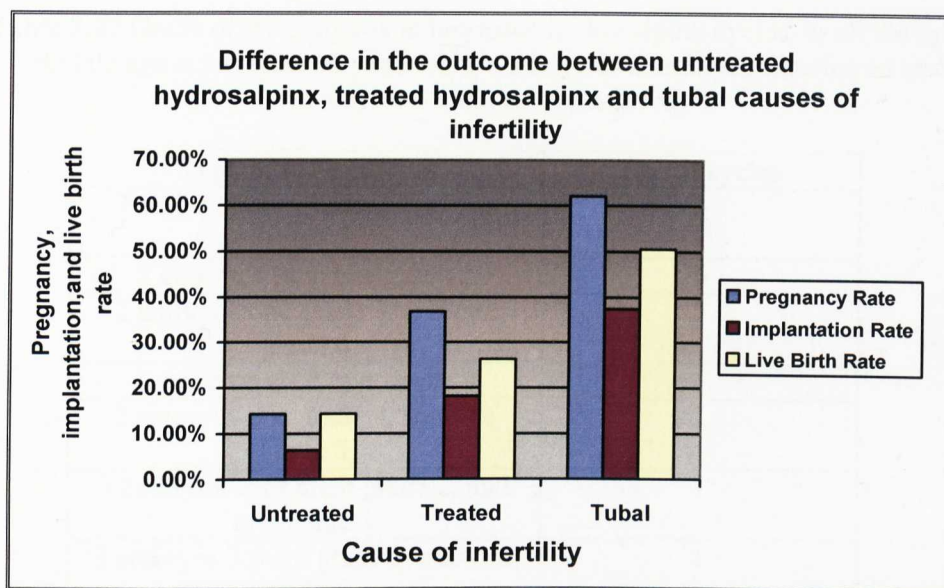
**Figure 2.21** Difference in the outcome of IVF, and IVF/ICSI, between cycles with treated hydrosalpinx, untreated hydrosalpinx, and tubal cause of infertility. This figure shows that the pregnancy, implantation, and live birth rates in the treated hydrosalpinx, and un treated hydrosalpinx cycles is low as compared to cycles with tubal cause of infertility, the difference is statistically significant.

### 2.5.15.1 Grade of embryos in hydrosalpinx patients

In our study we had 21 cycles in which the hydrosalpinx were untreated and there was transfer of two or three fresh embryos, and the female age was less than 36 years. There were 16 cycles of double embryo transfer, only 3/16 cycles (18.75%) of double embryo transfer were of low grade embryos (i.e. one embryo of grade 2, and one of grade 3). Furthermore, in 4/5 cycles of triple embryo transfer, there was at least one embryo of grade 4 or 5. This shows that around 80% of the embryos in the cycles of untreated hydrosalpinx are of good quality and suggests that the underlying cause for this low outcome is other than the quality of the embryo, see table (2.27).

**Table 2.27** Grade of the embryos in untreated hydrosalpinx cycles, in all the cycles
female age is less than 36 years old (in 16 cycles 2 embryos transferred and
in 5 cycle 3 embryos transferred)

| Type of the embryos | No. of cycles |
|---|---|
| 2 embryos one grade 2, and one grade 3 | 3 |
| 2 embryos each one grade 3 | 6 |
| 2 embryos one grade 3, and one grade 4 | 5 |
| 2 embryos each one grade 4 | 1 |
| 2 embryos one grade 4, and one grade 5 | 1 |
| 3 embryos, 2 of them grade 2, and one grade 3 | 1 |
| 3 embryos 2 them grade 3, and one grade 4 | 1 |
| 3 embryos, 2 of them grade 4, and one grade 3 | 1 |
| 3 embryos, graded 3, 4, and 5 | 1 |
| 3 embryos graded 2,3, and 4 | 1 |

# 2.6. Discussion:

## 2.6.1 Effect of female age on the outcome of IVF-ET:

IVF was developed in order to treat women who had blocked, damaged or
absent fallopian tubes. Subsequently it became a well established procedure for the
treatment of long standing infertility due to an ovulation, endometriosis, unexplained
infertility or certain types of infertility involving a male factor.

For a couple to undergo IVF the female partner should have functioning ovaries
and a normal uterus, and the male partner should have at least one sperm (Van
Steirteghem et al. 1993a). When sperm and eggs are mixed together in IVF, relatively
few sperms are used therefore it can be used when the male patient sperm quality is less
than ideal, although in cases of severe male factor infertility ICSI is recommended
(Palermo et al. 1992).

Research has shown that there is a fall in fertility with increasing female age and that this increase is first observed at 35 years of age, with a sharp increase after the age of 39 years and subsequently reaching almost complete inability to reproduce after the age of 44 (Tietze 1957).

Maternal age has been shown to adversely affect fecundity (Mencken et al. 1986), see figure (2.22). Maternal age has also been shown to have a negative effect on IVF success rates (Padilla and Garcia 1989). This research found that increased female age had a negative effect on the IVF success rate. These differences were shown to be more pronounced after the age of 36 years. The same conclusions were applied to the outcome of couples treated by IVF-ET. In our study, we have confirmed the relationship between advancing maternal age and reduced positive outcome of IVF-ET see table (2.4), and figure (2.4). At the same time, we did not find any significant effect of male age on the outcome of IVF-ET, see table (2.6), and figure (2.6). In both groups, we found that more than 70% of the embryos were of grade 3 and above, and according to the criteria of our lab that any embryo of grade 3 and above is regarded as a good quality. However, the pregnancy, implantation, and live birth rates still decrease and abortion rate increase as female age is increased. This means that it is likely that the defect is chromosomal and that we cannot detect it, or there is a defect in the endometrial receptivity which probably increasing with increase in female age. It is also worth noting that embryo morphology imperfectly reflects the viability of the embryo. It has been shown that embryos that may appear normal can contain chromosomal abnormalities and that the frequency of these abnormalities increased with the age of the women (Munne et al. 1995).
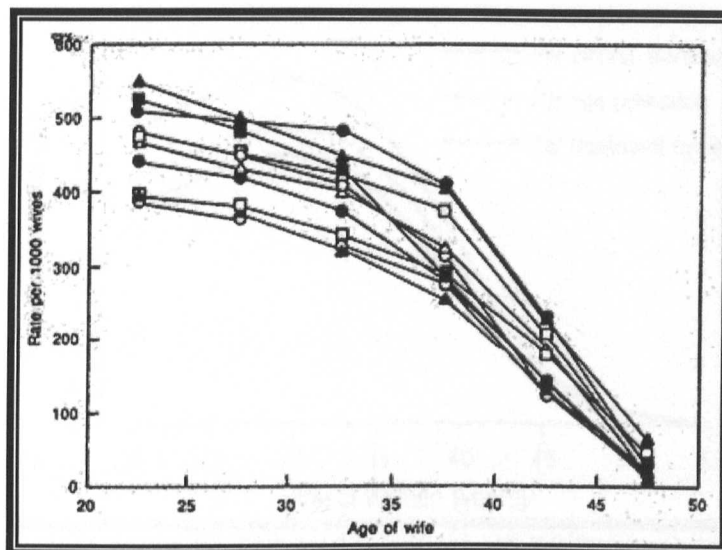
**Figure 2.22** The maternal fertility rate per maternal age. The fertile peak is when the woman is in her late teens, and early twenties. It begins to decline at the age of thirty, and drop more rapidly after the age of thirty five years. It plummets after age forty and pregnancy rate after age forty five is rare.
This figure is from [Mencken et al. (1986), with permission
from Science. Copyright (1986) APAS.]

Many studies have supported the widely held belief that age is the most important factor affecting the outcome of IVF-ET (Templeton et al. 1996). The effect of age on the outcome of IVF-ET is well illustrated by the information collected by the HFEA from all the IVF cycles carried out in UK, with results showing that the highest rate of live births was in the age group of 25-30 with younger women having a lower rate and a sharp decline in older women (Templeton et al. 1996), see figure (2.23).
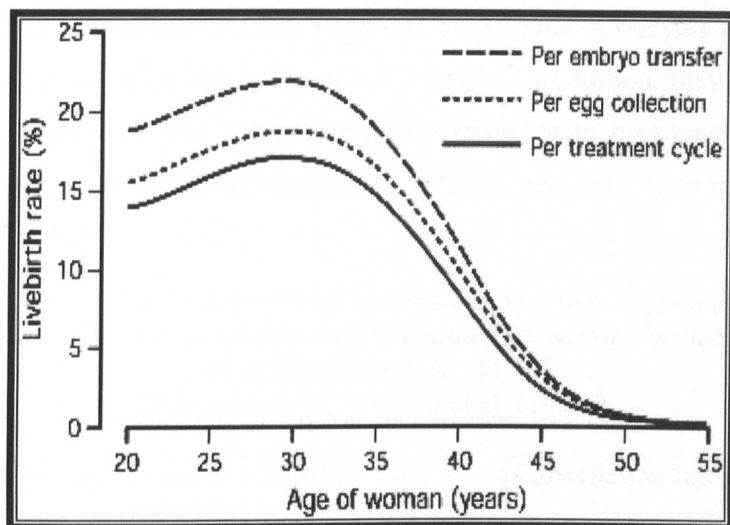
**Figure 2.23** Effects of the female age on the live birth rate per cycle, per egg collection and per embryo transfer. The authors found that younger women had lower rate with the highest rate of live births in the age group of 25-30, and then there is a sharp decline in the older age group.
[This figure is from Templeton et al. (1996), the Lancet]

FSH levels have been shown to be more sensitive than the women's chronological age in determining her likely ovarian responsiveness to stimulation (Cahill et al. 1994). Lashen and co workers did a retrospective study, and they found that patients who received 225 or 300 IU follicle-stimulating hormone in their first cycle of IVF showed a similar response in their second cycle, except for patients who had significantly high oestradiol levels, and they concluded that exceeding the daily dose of 300IU is unrewarding (Lashen et al1998). Sharif et al found that LH levels and menstrual cycle pattern were unhelpful in determining the same result (Sharif et al. 1998).

A study of 344 infertile patients who had IVF, to examine the relative effect of basal (FSH) concentration and the woman's age on predicting the ovarian response to gonadotrophin stimulation, normal fertilisation rate and pregnancy rate in IVF, showed that increasing basal FSH concentration but not increasing age were associated with a significantly increased cancellation rate (Sharif et al. 1998). Both increasing basal FSH and increased age were associated significantly with increased total gonadotrophin dose and reduced numbers of oocytes collected and pregnancy rates. Furthermore, the

authors found the association of basal FSH with the number of oocytes was significant, independent of, and stronger than the effects of age. In addition, they found that the age, but not basal FSH, was independently associated with pregnancy rate. Neither basal FSH, nor age had significant association with normal fertilisation rate (Sharif et al. 1998) see table (2.28).

**Table 2.28** Effect of increased FSH and increasing female age on predicting ovarian response to gonadotrophin stimulation, normal fertilisation rate and pregnancy rate in IVF.
[This table is from Sharif et al. (1998)]

| Increasing the basal FSH associated | ↑Cancellation rate |
|---|---|
| Increasing basal FSH and increasing the female age associated | ↑ Gonadotrophin dose ↓ in the oocyte collected ↓ in the pregnancy rate |
| Neither the ↑FSH nor the age had significant association with | Normal Fertilization rate |

It has been found that infertile patients who develop fewer than three dominant follicles, defined as follicles with a diameter greater than 17mm, measured on ultrasound, on the day of intended hCG administration, have an almost universally poor outcome, whatever the level of FSH (Lavery et al. 1998).

Spandorfer et al. (1998) found a significant decline in the number of oocytes retrieved and the number of mature oocytes obtained with advancing maternal age. In addition, the authors found an increase in the occurrence of digyny with parental ageing, while no difference in single or bipronuclear fertilization was found. Furthermore, they found that older women had a decreased incidence of single pronuclei formation and an increase in digyny, but no significant difference in the percentage of oocytes that underwent two-pronuclear fertilization.

Hassan and Killick (2003) in a study of resolved sub-fertility found that women more than 35 years old were more likely to be sub-fertile than women less than 25 years. However, it has been shown that reproductive age is different from the temporal age, instead depending on ovarian size, ovarian volume, ovarian reserve and remaining time until menopause (Hamish and Thomas 2004).

Once the ovarian volume, ovarian size, decrease in ovarian reserve and time to menopause are known, it is possible to calculate a woman's reproductive age, which may be younger or older than her actual age (Hamish and Thomas 2004), see figure (2.24).
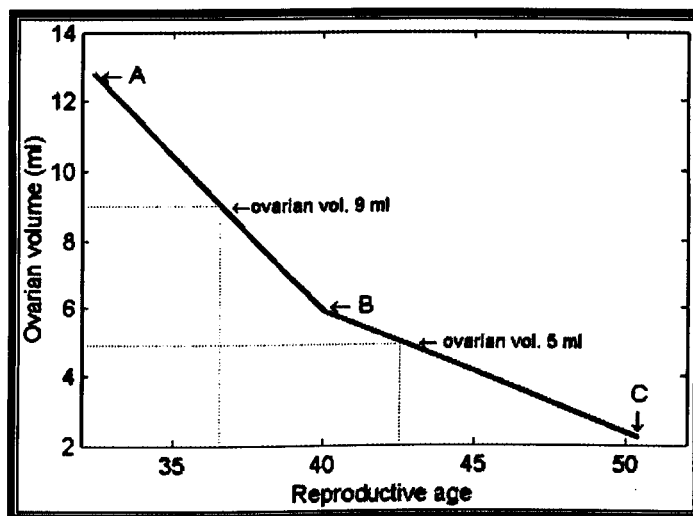


**Figure 2.24** Reproductive age (years) versus ovarian volume (ml) for 40-year-old women.. This figure shows that when two individuals aged 40, with mean ovarian volume of 9 and 5 ml respectively, giving a reproductive age of 36 and 42 respectively. This means that reproductive age depends on the ovarian volume
[This figure is from Hamish and Thomas (2004); Hum. Reprod].

Lane et al. (2006), found that Clinical pregnancy rates increase until age 30 [OR 1.72 , and 95% CI (1.19–2.49)] before demonstrating a linear decline.

## 2.6.2 Effect of the male age on the outcome of IVF-ET:

A retrospective study to determine the effect of age on sperm fecundability using oocyte donation as an in vivo model suggested that male age does not have an impact on the outcome of IVF-ET (Gallardo et al. 1996). All the oocytes were obtained from patients less than 35 years of age and the semen samples were divided into four groups according to male age. The authors found that the sperm characteristics were

similar in fresh samples as well as after preparation for IVF among males of different ages. In addition, they found that fertilization, embryo quality, pregnancy and implantation rates were similar among all the established groups. The authors therefore concluded that male age (up to 64 years) does not affect sperm characteristics or its ability to fertilize human eggs. Similarly, embryo development in vitro, as well as implantation in recipient uteri, is not affected by the age of the male providing the semen sample (Gallardo et al. 1996).

Another retrospective study of 821 consecutive ICSI cases found that there is a significant linear decline in semen volume but no significant differences in the concentration, motility or morphology of the spermatozoa with paternal ageing. Moreover, the authors concluded that pregnancy outcomes were not influenced by the age of male partner, while a strong negative correlation was found with maternal ageing (Spandorfer et al. 1998). In our study, we did not find any significant effect of male age on the outcome of IVF-ET. In 2003, Hassan and Prof. Killick did a questionnaire study, evaluating the effect of male age on time to pregnancy. They found that increasing male age was associated with a significantly increased time to pregnancy and declining conception rates, and there was five fold increases in time to pregnancy with male age more than 45 years. De la Rochebrochard et al. (2006) found that odds ratio of failure to conceive for paternal age $\geq$ 40 years was 2.00 (95% CI 1.10-3.61) when the woman was 35-37 years old. The OR was 2.03 (95% CI 1.12-3.68) for age 38-40 years, and 5.74 (95% CI 2.16, 15.23) for age 41 years and over. They concluded that paternal age over 40 years is an important risk factor for failure to conceive.

## 2.6.3 Embryo cryopreservation:

Embryo cryopreservation is a process of freezing, storing, and thawing embryos. It can enhance pregnancy rates by allowing excess embryos not replaced in a fresh embryo transfer to be stored for future use. Some couples do not like the idea of destroying embryos simply because they are "left over" from an IVF cycle, others know or suspect that they will need to have further IVF cycles in the future and prefer to freeze their embryos in order to make future IVF cycles less stressful physically for the female.

Indications for cryopreservation are: (1)Risk of ovarian hyperstimulation syndrome (OHSS), (2) Poor quality endometrium "a thin uterine lining", (3) Intermenstrual bleeding, (4) Planned "banking" cycle in which the patient elects to store all embryos. Embryos of sufficient quality that are not transferred can be cryopreserved (Edwards and Brody 1995).

Embryos can be cryopreserved at various times after fertilization, ranging from one day after fertilization up to five or six days after (Van Steirteghem et al. 1994a). The embryologist will select embryos that are suitable for freezing. Embryos that are ideal for freezing have blastomeres of equal size and display minimal or no fragmentation (Van Steirteghem et al. 1992).

The intention of cryopreservation is to prevent all chemical reactions from taking place and this target is achieved when cells reach the temperature of -130°C but they are usually stored in liquid nitrogen at -196°C. The main problem is reaching -196°C without damaging the cells, and there is approximately a 30% chance of damage following the cryopreservation of embryos. A correct balance between the freezing rate and the cryoprotective concentration ameliorates this damage (Ashwood and smith 1986).

The quality of embryos undergoing cryopreservation is a major determinant of survival. Depending on the stage of embryo development, frozen embryos are thawed for one day before the transfer (Edwards and Brody 1995).

Since 1988, a standard freezing–thawing protocol, based on propandiol as cryoprotectant, has been used (Testart et al. 1986) in the Hull IVF Unit.

Studies have been done to assess the outcome of frozen cycles. It has been shown that the pregnancy rate in frozen cycles depends on the number of oocytes retrieved. The authors also found that the 'take-home baby rate' per stimulation cycle was 28.3% when 6 – 10 oocytes were retrieved as compared to 41.5% when more than 10 oocytes were retrieved (Toner et al. 1991a).

Two further studies reported the pregnancy rate following frozen embryo transfer to be 24-30%, and 15-20% respectively (Lin et al. 1995; Ozer and Vermesh

1999). Selick et al. (1995) found that implantation rates per embryo 12.6% and 8.1%, and live birth rates per transfer 26.2% and 13.3% for fresh and frozen transfers respectively. Significant differences were not found despite the larger number of high quality embryos transferred during the fresh cycles. The authors therefore concluded that embryo cryopreservation adversely affects embryo quality, but does not have detrimental effects on the implantation or pregnancy potential of high quality embryos. Because of the loss of embryos during the freezing and thawing process involved in frozen embryo cycles they suggested that every effort should be made to attempt a fresh transfer wherever possible (Selick et al. 1995).

Other authors have suggested that there is no difference between frozen embryo transfer in natural and hormone replacement cycles as regards pregnancy rates per cycle 26% and 25%, ongoing pregnancy/delivery rate 20.8% in both groups, and implantation rate 10.3% and 10.6% (Al Shawaf et al. 1993a). In addition, the authors found that pregnancy rates were not influenced by the number of frozen embryos transferred or the stage at which the embryos were cryopreserved. However, the pregnancy rate was low (7.4%) if the embryos had less than three blastomeres and if the fragmentation was greater than 50% (0% pregnancy rate). The authors also found that in the hormone replacement cycles, age did not influence the outcome and women 40 years and older had a pregnancy rate of 29.4% per cycle. Other important findings were that no pregnancy resulted from FET (Frozen embryo transfer) in natural or hormone replacement cycles when the endometrium was thin (thickness less than 8 mm and grade C). Furthermore, they found that the pregnancy rates were higher when the endometrium was greater than or equal to 8 mm thickness and grade B (42.4%) or grade A (21.2%). The authors concluded that FER outcomes in natural cycles were similar to those arising with hormone replacement therapy, provided good selection criteria were used, and they suggested that vaginal ultrasonography can assist in timing the day of replacement and in identification of cases to be cancelled before the transfer (AL Shawaf et al. 1993a).

In our study, we found that the pregnancy and implantation rate after the transfer of 2 fresh and 2 frozen embryos were (37.3%, versus 27.3%) and (23.2%, versus 16.36%) respectively, P >0.05, see table (2.9), figure (2.7). We can see that the

outcome is lower with frozen cycles but the difference was not statistically significant, with higher number of frozen cycles we may approach to a statistically significant difference. In our retrospective analysis, we found that there is no effect due to the number of transferred embryos in the frozen cycles, see table 10(a), (b), figure (2.8), while in the fresh cycles there is a statistically significant difference according to the number of embryos transferred, with better outcomes with 2 embryo transfers. While in one embryo transfer and three embryo transfers the outcomes were not good, see table 2.11(a), and figure (2.9). This may be because the only patients with a history of multiple IVF failures had three embryos transferred. At the other end of the spectrum single embryo transfers were not done by choice but were instead performed after the woman stimulated poorly due to a diminished ovarian reserve or was performed for patients who had many oocytes collected but where there was a defect in the fertilization process due to the presence of male factor or unexplained causes. We will talk more about the effects of the number and the quality of embryos on the outcome of IVF and compare our result with the result of other studies.

## 2.6.4 Effect of the number of embryos transferred on the outcome of IVF-ET:

European and American registries of medically assisted reproduction indicate high multiple pregnancy rates after IVF/ ICSI, around 25% for twin and 3-5% for high order multiple pregnancies (European IVF monitoring program 2002). An excellent review of this problem that was carried out by an expert group consisting of both Europeans and Americans, made a recommendation that triplet pregnancies should be avoided and that twin pregnancies should be reduced (Gerris and Neubourg 2005).

Although previous studies have shown that pregnancy rates increase steadily as more embryos are replaced, implantation rates may reach 40% or more in some patients with three replaced embryos (Pandian et al. 2004), but from our study we can see that the pregnancy rate after 3 embryo transfer is lower than the pregnancy rate after 2 embryo transfer, see table 2.11(a), (b), figure (2.9). The likely explanation for that is that most of the patients who had ET of 3 embryos are patients with unfavourable prognosis, such as patients who are above forty or patients who had previous recurrent IVF failure. Therefore, our conclusion is that if results are already good for two

embryo transfer then in the future, as equipment and techniques improve results will be good for single embryo transfers too, with less risk of high order multiple pregnancies.

There are many randomised studies comparing the transfer of one embryo and the transfer of two embryos. Research by Gerris et al. (1999) showed that the pregnancy rate after single embryo transfer and double embryo transfer were 38.5% and 74% respectively and the twin pregnancy rates were 10% and 30% respectively. A different group of researchers found that the pregnancy rates were 32.4% and 47.1% respectively, and that the twin incidences for single and for double transfers were 0.4% and 18.4% respectively (Martikainen et al. 2001).

A third group of authors found that in their study, that the pregnancy rate for a single embryo transfer was 60.9%, and for the double embryo transfers was 76% (Gardner et al. 2004). The authors also showed that there were no twin pregnancies amongst the single embryo transfers while in the second group the twin pregnancy rate was 47.4%, see table (2.29).

**Table 2.29** Previous randomised studies comparing between single with double embryo transfer. [This table is from Gerris (2004)]

| The study | Total cycles | One ET pregnancy rate | Twin pregnancy rate | Double ET Pregnancy rate | Twin pregnan cy rate |
|---|---|---|---|---|---|
| Gerris et al. 1999 | 53 | 10/26 (38.5%) | 10% | 20/27 (74%) | 30% |
| Martikaiine n et al. 2001 | 144 | 24/74 (32.4%) | 0.4% | 33/70 (47.1%) | (18.2%) |
| Gardner et al. 2004 | 48 | 14/23 (60.9%) | 0% | 19/25 (76%) | 9/19 (47.4%) |

Lane et al. (2006) found that The odds of achieving a successful clinical pregnancy with IVF are greatest with retrieval of approximately 20 oocytes, transfer of no more than 2 embryos, and the development of about five 2 pronuclei embryos in women <37 years old and ten 2PN embryos in women ≥37 years old.

Reducing the number of embryos for transfer will not entirely eliminate multiple pregnancies. Monozygotic twinning has been found to be higher following

assisted reproduction (Edwards et al. 1986) especially in the subgroup of ART patients whose embryos have been zona manipulated either for assisted fertilization (SUZI or ICSI) or assisted hatching (Slotnick and Ortega 1996). Others, however, have reported that the frequency of monozygotic twinning is not different for patients whose embryos have been zona manipulated and those that remain zona intact (Sills et al 2000). The incidence of monozygotic twinning has also been reported to be very high following blastocyst transfer (Rijnders et al. 1998).

In our analysis, we found that there is a statistically significant difference in the pregnancy rate between a single embryo transfer 16.9% and a double embryo transfer 37.3% or triple embryo transfer 27.12% (P <0.05), see table 2.11(a),(b), figure (2.9). In addition, the incidence of twin and triplet in these 3 groups were (0%, 0%), (24.5%, 0.02%), and (26.86%, 1.5%) respectively, see table 2.11(c), figure (2.10). According to this result we can conclude that single embryo transfer should be recommended for patients with good prognosis, such as those who are young with a good number of fertilised oocyte that allow proper selection of top quality embryos for transfer and where the rest of the embryos will be frozen for use in the future to avoid the risk of ovarian hyperstimulation. Equally, for patients above 36 it would be better to continue double embryo transfer in order to avoid low pregnancy rates.

## 2.6.5 Effect of the embryo quality on the outcome of IVF-ET (embryo grade and the cleavage rate):

In human IVF, the selection of a good embryo, i.e. one that should implant and give rise to a baby remains a major issue. Most IVF programmes select transferred embryos on their morphological appearance on the day of the transfer using several criteria have been shown to correlate with embryo viability, including the number of blastomeres, regularity of the cells and the presence or absence of cytoplasmic fragmentation (Staessen et al. 1992), see figure (2.25).

(A) After Insemination/ICSI
(B) Zygote morphology 16-18 hours - the number and Distribution of nucleolar precursor bodies (NPB) and the existence of cytoplasmic halo
(C) The early cleavage of zygotes at 25–27 hours after insemination/ICSI

(D) Cleavage stage embryo quality on day 2 or 3 — The number of blastomeres and the embryo morphology (fragmentation, blastomere shape and multinucleation) all these are important criteria for the selection of embryos for transfer

(E) Blastocyst — the size Of inner cell mass And the cohesiveness Of trophectoderm

**Figure 2.25** Preimplantation embryo development and important aspect of embryo selection for transfer. [This figure is from Andres Salumets (2003)]

Evidence shows that severely fragmented embryos had a lower ability to develop to a blastocyst stage, and that the presence of the cytoplasmic fragmentation is associated with reduction in the implantation rate (Hardy et al. 1989; and Staessen et al. 1992). Other evidence has suggested that poor embryo morphology is associated with chromosomal abnormalities. Almeida and Bolton (1998) showed that 63.4% of embryos that arrest between the pronucleate and the eight-cell stage are chromosomally abnormal. Magli et al. (1998) found that slow cleaving (two to six cells on Day 3) and rapidly cleaving (nine or more cells on Day 3) embryos show a higher incidence of chromosomal aneuploidy than those embryos showing normal cleavage kinetics (seven to eight cells on Day 3). Furthermore, it was found that the chance of live birth is

related to the number of fertilized oocytes because of the greater selection of the embryo (Templeton et al. 1998).

A retrospective study looking at the outcomes of IVF-ET according to the grade and the number of the embryos used found that the pregnancy rate was 11.9% for the single transfers, 19% for the double transfers, and 34.1% for the triple transfers (Staessen et al. 1992). 31% of these triple embryo replacements resulted in a multiple gestation, see table 2.30(a). They also found that embryos that had undergone at least two mitotic divisions at 44 to 48hrs implanted better than two-cell embryos of comparable morphological appearance, implantation rate per transferred embryo was [21.3% versus 12.3%( $P < 0.001$)], see table 2.30(b). The authors also found that heavily fragmented embryos did not implant as well as embryos with fewer anucleate fragments [(1.5% versus 14.1%, $P < 0.001$)], see table 2.30(c).

**Table2.30 (a)** Difference in outcome according the number of embryos transferred.
[This table is from Staessen et al. (1992)]

|  | Single ET | Double ET | Triple ET | Risk of twin after Triple ET |
|---|---|---|---|---|
| Pregnancy rate | 11.9% | 19% | 34.1% | 31% |

**Table 2.30(b)** Difference in the outcome according to the number of blastomeres of each embryo at the time of embryo transfer.
[This table is from Staessen et al. (1992)]

|  | Embryo undergone two mitotic divisions at 44 to 48hrs | Two-cell embryos at the time of ET | P |
|---|---|---|---|
| Pregnancy rate | 21.3% | 12.3% | <0.001 |

**Table 2.30(c)** Pregnancy rate according to the level of fragmentation.
[This table is from Staessen et al. (1992)]

|  | Heavily fragmented embryos | Embryos with little fragmentation | P |
|---|---|---|---|
| Pregnancy rate | 1.5% | 14.1% | 0.001 |

The authors concluded that pregnancy rate, implantation rate, and the incidence of multiple pregnancies increased significantly with the number of good quality embryos that were transferred (Staessen et al. 1992).

Research has compared the pregnancy rates in two groups of patients on day two of embryo transfer (Carrillo et al. 1998). In one group the number of blastomeres was greater than 4 cells, and in the second group the number of blastomeres was less than 4 cells. Results showed that the pregnancy rate in both group was 31% and 11% respectively. In addition, they compared the pregnancy rate on day three embryo transfers between two groups, in one group the number of blastomeres was greater than 8, and in the second group the number of the blastomeres was less than 8. The authors found that the pregnancy rate was 53% and 23% respectively (Carrillo et al. 1998), see table 2.31(a).

A prospective randomised study of 110 women who were less than 34 years of age compared the transfer of single top quality embryo with double top quality embryo (Gerris et al. 1999). A top quality embryo was characterized by the presence of 4 or 5 blastomeres at day 2 and at least 7 blastomeres on day 3 after insemination, plus the absence of multinucleated blastomeres and less than 20% cellular fragments on day 2 and day 3 after fertilization. The authors found that the implantation rate and the ongoing pregnancy rate after transfer of top quality single embryos was 42.3% and 38.5% with one monozygotic twin, while in case of double embryo transfer the implantation rate and the ongoing pregnancy rate was 48.1% and 74% respectively. Out of 20 ongoing pregnancies there were 6 sets of twins (30%). Therefore, the authors concluded that by using single embryo transfer and strict embryo criteria an ongoing pregnancy rate similar to that in normal fertile couples can be achieved after IVF/ICSI, while limiting the dizygotic twin pregnancy rate to its natural incidence of less than 1% of all ongoing pregnancies (Gerris et al. 1999).

In 2000, a study looked at the importance of the grade of the embryos selected for transfer in 380 fresh embryo transfer cycles according to the conventional criteria (Tesarik et al. 2000). The authors found that the transfer of only those embryos that developed from zygotes judged normal at the pronuclear stage (pattern 0) gave significantly higher pregnancy (44.8%), and implantation (30.2%) rates compared with

the pregnancy (22.1%; P < 0. 05) and implantation rates (11.2%; P < 0.001) for the transfers of only those embryos that developed from zygotes judged abnormal (non-pattern 0). Furthermore, they found that the transfer of only one pattern 0 embryo was sufficient for the optimal chance of pregnancy (no differences in pregnancy rates after transfer of one, two or three pattern 0 embryos), whereas the transfer of two pattern 0 embryos mostly resulted in a twin pregnancy. The authors suggested including the criteria based on pronuclear morphology and that it may lead to the application of a single embryo transfer policy and optimization of the process for the selection of embryos for transfer and cryopreservation (Tesarik et al. 2000), see table 2.31(b).

**Table 2.31(a)** Effect of the number of the blastomeres on the outcome of IVF-ET.
[This table is from Carrillo et al. (1998)]

|  | Day 2 embryo >4cell | Day 2 embryo <4 cell | Day 3 embryo >8cell | Day 3 embryo <8 cell |
|---|---|---|---|---|
| Pregnancy rate | 31% | 11% | 53% | 23% |

**Table 2.31(b)** Effect of the morphology of the embryo at the pronuclear stage.
[This table is from Tesarik et al. (2000)].

|  | 0 pattern embryos (normal) at pro nucleus stage | Abnormal embryos at pro nucleus stage | P |
|---|---|---|---|
| Pregnancy rate/ET | 44.8% | 22.1% | < 0.05 |
| Implantation rate | 30.2% | 11.2% | < 0.001 |

All of these previous studies appear to support the findings of our study in that higher grade embryos with higher rates of cleavage have result in a better chance of pregnancy. These findings may influence us to change our policy of embryo transfer from double embryo transfer to single embryo transfer in patients with a good prognosis.

## 2.6.6 Day 2 versus day 3 embryo transfer:

Embryos may be transferred to the patient on Day 1 post insemination at the zygote stage of development by (PROST "Pronuclear stage transfer" or ZIFT "Zygote Intrafallopian Transfer"), on Day 2 as two-cell to four-cell early cleavage-stage embryos by (ZIFT, TEST "Tubal embryo stage transfer", TCET, or TMET), same can be done on Day 3 as six-cell to eight-cell early cleavage-stage embryos , on Day 4 as morula (ET), or on Days 5 to 7 as blastocyst of varied morphology (ET). (Jones et al ©www.who.int/reproductive-health/infertility/20.pdf Accessed on 24/09/06 at 3:43 pm)

One of the disadvantages of transferring early zygotes is the inability to select the most viable zygotes from a large cohort for transfer. However, high pregnancy and implantation rates have recently been reported for pronuclear stage transfer when zygotes are selected for transfer according to certain pronuclear morphological features (Tesarik et al. 2000).

A retrospective study of 176 IVF-ET patients found that day 3 embryo transfers are associated with a significant increase in the implantation and pregnancy rates and delaying the embryo transfer to day 3 permits the selection of more viable embryos than is possible on day 2 (Carrillo et al. 1998), see table 2.32(a).

A randomised prospective study of 329 embryo transfers which resulted in 106 clinical pregnancies (32.2%) showed that pregnancy rate achieved were 20% on day one, 30.4% in day 2 and 3, 50% on day 4 and 5 (P=0.03) (Markus et al. 2003). The authors concluded that embryo transfer performed on day 4 or 5 enhanced the rate of pregnancy significantly compared to transfer on day 1, 2 or 3, see table 2.32(b).

**Table 2.32(a)** Day 2 versus day 3 embryo transfer.
[This table is from Carrillo et al. (1998)]

|  | Implantation rate | Pregnancy rate |
|---|---|---|
| Day two embryo transfer | 13% | 26% |
| Day three embryo transfer | 24% | 44% |

**Table 2.32(b)** Difference in the outcome according to the day of embryo transfer. [This table is from Markus et al. (2003)]

| Day of embryo transfer | Day one | Day two and day three | Day four and day five | P value |
|---|---|---|---|---|
| Pregnancy rate | 20% | 30.4% | 50% | P=0.03 |

In the paper by Papanikolaou et al. (2005) blastocyst-stage transfer resulted in a significantly higher ongoing pregnancy rate (51.3 versus 27.4%; OR 2.78, 95% CI 1.45-5.34) and live birth rate (47.5 versus 27.4%; OR 2.40, 95% CI 1.25-4.59) compared with day 3 embryo transfer when at least four embryos are available on day 3 of embryo culture. Blake et al. (2005) found that there is no evidence of a difference in live birth or pregnancy outcomes between Day 2 to 3 and Day 5 to 6 transfer of embryos, in addition they found that blastocyst transfer was associated with an increase in failure to transfer any embryos in a cycle and a decrease in embryo freezing rates.

In our study, we found that there is a statistically significant difference in the pregnancy and implantation rate between day 2 embryo transfers and day 3 embryo transfers (35.10%, versus 42.8%) P<0.024, and (21.53%, versus 27.43%) P< 0.005 respectively, see table (2.20), figure (2.14). Although our result might be biased because embryo transfer was done according to the day of egg retrieval, but still we conclude that delaying embryo transfer for one day may increase the pregnancy rate by allowing optimum selection of the embryo for transfer.

## 2.6.7 Effect of easy or difficult embryo transfer on the outcome of IVF –ET:

Studies have been done comparing the outcome of difficult and easy procedures of embryo transfer on the outcome (Leeton et al. 1982; Wood et al. 1985; Egan et al. 1990; Mansour et al. 1990; Tur Kaspa et al. 1998; and Lesny et al. 1999a). Some of the studies show that difficult embryo transfer did not affect the pregnancy outcome (Wood et al. 1985; Egan et al. 1990; and Tur- Kaspa et al. 1998), while other studies showed that difficult embryo transfer negatively influences the outcome (Leeton et al. 1982; Mansour et al. 1990). In our study, there was no statistically significant difference in

the outcome and therefore our results appear to support the studies that showed that there is no difference in the pregnancy and implantation rate between easy and the difficult TCET, (37.3%, versus 40%) and (23%, versus 26.3%) respectively, see table 2.21(b), figure (2.15). The underlying cause for this result probably due to embryo transfer procedures are not as difficult as those shown in the other studies. Our results also show that the outcome after TMET is low at 10%. An explanation of this may be that even if the TMET is easy it may initiate the development of junctional zone contractions. This suggestion would appear to be supported by work that found that after the insertion of a Towaco needle there was a significant increase in the number of random, opposing and cervico-fundal contractions (Biervliet et al. 2002). In the next chapter there will be more discussion about these studies.

## 2.6.8 Effect of the cause of infertility on the outcome of IVF-ET:

### 2.6.8.1 Hydrosalpinx

Previous studies have shown an impaired outcome of IVF in the presence of hydrosalpinges, see table (2.33).

**Table 2.33** Previous studies showing the difference of the Pregnancy rate after IVF-ET in patients with and without hydrosalpinx. [This table is from Zeyneloglu et al. (1998)]

| The study | hydrosalpinx group | | Group without hydrosalpinx | |
|---|---|---|---|---|
| Sim et al. 1993 | 43/234 | (18.37%) | 341/1287 | (26.49%) |
| Andersen et al. 1994 | 9/91 | (9.8%) | 224/744 | (30.1%) |
| Strandell et al. 1994 | 12/91 | (13.18%) | 74/285 | (25.96%) |
| Blazer et al. 1997 | 35/155 | (22.58%) | 105/185 | (56.75%) |
| Van Dromme et al. 1995 | 7/69 | (10.14%) | 14/61 | (22.95%) |
| Sharara et al. 1996 | 10/30 | (30%) | 11/24 | (45.83%) |
| Akman et al.1996 | 1/14 | (7.1%) | 24/98 | (24.5%) |

Salpingectomy has been suggested as the most radical treatment and other options, such as salpingostomy and transvaginal aspiration of the fluid from the hydrosalpinx, have also been discussed. Salpingectomy was tested and found to be of benefit in patients with hydrosalpinx large enough to be visible on ultrasound (Strandell et al. 1998; 1999). Surrey et al. (2001) did not approach a statistically significant

difference in the clinical pregnancy and implantation rates between 2 groups of patients in which the hydrosalpinx was treated before IVF cycle. In the first group the hydrosalpinx was treated with salpingectomy, while in the second group the hydrosalpinx was treated with bipolar proximal tubal occlusion. The clinical pregnancy and the implantation rates for the first and the second groups were 57.1%, 29.2 +/- 5.9%, respectively, and 46.7%, 19.4 +/- 6.1%, respectively.

A postal survey of hydrosalpinx management prior to IVF in the United Kingdom was done by Hammadie et al. (2004) to determine the policy for the management of hydrosalpinx in infertile women prior to IVF treatment. They found that, there were 75% responders, of which 91% indicated that they discussed the effect of hydrosalpinx on IVF outcome. 12% did not recommend treatment of hydrosalpinx prior to IVF treatment, while 36%, 33% and 19% recommended treatment weakly, strongly and very strongly respectively. The treatment options offered by clinicians were laparoscopic salpingectomy 75%, open salpingectomy 45%, salpingostomy 40%, proximal tubal occlusion 34%, transvaginal sonographic (TVS) aspiration during oocyte collection 23% and TVS aspiration before oocyte collection 10%. Only 28% of the responders had a protocol or guidelines for the management of hydrosalpinx. They concluded that more attention should be given to patients with hydrosalpinx prior to IVF treatment and patients should be counselled about the negative effect of hydrosalpinx on IVF outcome. Further more they found that there is a wide variation in the management of hydrosalpinx prior to IVF treatment in the UK and many treatment options may be questionableas they are not yet based on evidence.

In our data if we compare the relationship between the hydrosalpinx and the grade of 2 embryos we found that the embryo grades were very low in 9 cycles (14.06%), and in the remaining cycles (85%) the embryos are grade 3 and above. Even when three good quality embryos were transferred, pregnancy rate was low which would seem to suggest that the negative effect of hydrosalpinx in our study is related not only to the low quality embryo but also to other causes such as impaired endometrial receptivity, which may be the underlying cause for low pregnancy and implantation rates in patients with hydrosalpinx. Other interesting results that we found suggest that the pregnancy and implantation rate is higher in the treated hydrosalpinx

cycles 36.84% and 18.18% respectively, while in the untreated cycles the pregnancy and implantation rates were 14.3% and 6.36% respectively, see table 2.26(b), and figure (2.21). This result supports the studies that show that treating the hydrosalpinx with salpingectomy will improve the pregnancy and the implantation rate, see table (2.33).

### 2.6.8.2 Endometriosis:

IVF–ET has become a common method of treating fertility problems in cases of endometriosis. However, studies show that cases of endometriosis have lower chances of success with IVF-ET than tubal or unexplained causes of infertility (Wardle et al.1985; Mills et al. 1992; Fleming et al. 1994).

Hull et al. (1998) suggested that endometriosis is associated with oocyte and follicular dysfunction. This is supported by reduced fertilization rates observed in in-vitro fertilization treatment using various stimulation regimens (Wardle et al. 1985; Mills et al. 1992; Fleming et al. 1994). Other authors have put forward the explanation that these low rates may be related to the mechanisms of angiogenesis, the immune system, and/or endometrial receptivity (Toya et al. 2000).

It has been found that fertilization rates are significantly lower in women with endometriosis, but the cleavage, implantation, and pregnancy rates do not differ (Bergendal et al. 1998).

Toya et al. In a work published in 2000, they collected granulosa cells from the follicular fluid of patients with endometriosis and they found that the rate of apoptosis and S phase was significantly higher in patients with endometriosis (Toya et al. 2000). Beyond that the percentage of G2/M-phase was significantly lower in the endometriosis group when compared to the other causes of infertility and the authors therefore concluded that patients with endometriosis have an abnormality in the cell cycle and a higher incidence of apoptotic cells in their granulosa cells, see figure (2.26). These phenomena might be associated with this patient group's poor outcome after treatment with IVF.
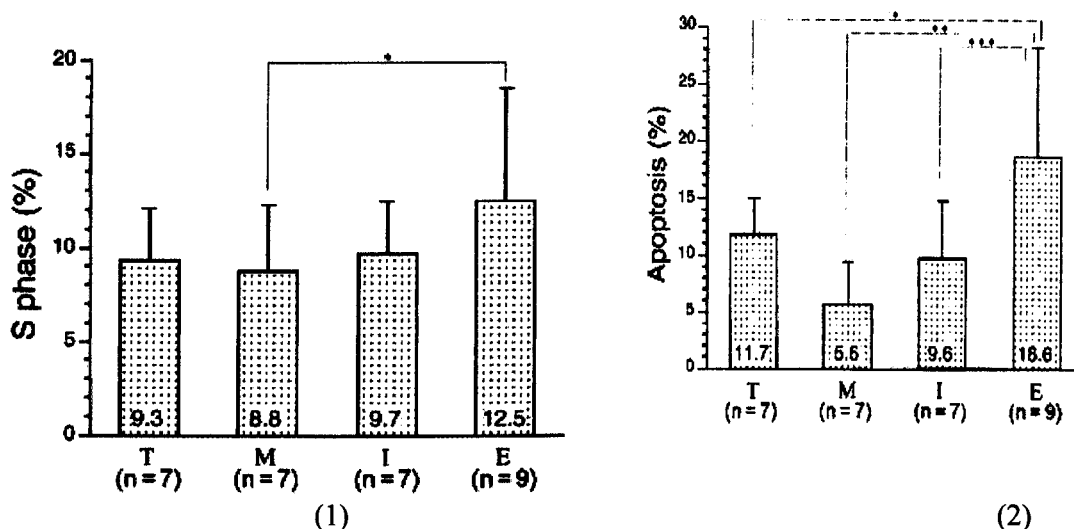
**Figure 2.26**The rate of apoptosis and S phase granulosa cell in endometriosis

(1) the rate of S phase granulosa cell in the endometriosis group was higher than the other groups; there was statistically significant difference between endometriosis and male factor group.

(2) Endometriosis group have the highest level of apoptosis in the granulosa cells as compared to the cases with unexplained infertility (Idiopathic), cases with male factor infertility, and cases with tubal infertility.
[This figure is from Toya et al. (2000), Fertil. Steril.]

A 2005 study by Kuivasaari and co-workers evaluated the impact of moderate to severe endometriosis on cumulative IVF outcome . In this study, endometriosis was diagnosed by laparoscopy or laparotomy and classified as minimal to mild endometriosis or moderate to severe endometriosis according to the (American Society for Reproductive Medicine's 1997 system of classification). The reference group consisted of women with tubal infertility and the researchers found that there is a significantly lower pregnancy rate per fresh embryo transfer amongst women of stage 3 and 4 with endometriosis (22.6%), as compared to stage 1 and 2 endometriosis (40%) or tubal infertility (36%). The cumulative pregnancy and live-born rates resulting from four IVF treatments without frozen embryo transfers were 64.5% and 51.6% in women with stage I/II endometriosis, 44.8% and 32.8% in women with stage III/IV endometriosis, and 69%, 40.2% in the reference group respectively. With frozen embryo transfers included, the equivalent rates were 67.7% and 55.8% in women with

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stage I/II endometriosis, 56.7% and 40.3% in women with stage III/IV endometriosis and 81.6% and 43.7% in the reference group respectively. The authors therefore concluded that patients with stage 3 and 4 endometriosis have a lower chance of pregnancy compared to mild cases of endometriosis and tubal causes of infertility; see table 2.34 (a) and (b), figure (2.27).
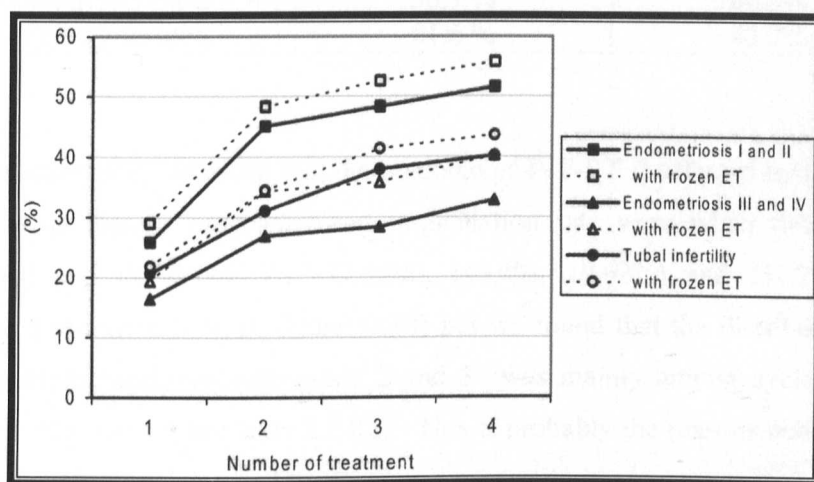


**Figure 2.27** The cumulative pregnancy and live born rates in endometriosis. This figure shows that there is a significantly lower pregnancy rate per fresh embryo transfer amongst women of stage 3 and 4 with endometriosis, as compared to stage 1 and 2 endometriosis, or tubal infertility
[This figure is from Kuivasaari et al. (2005)]

**Table 2.34(a)** Cumulative pregnancy and live-born rates of fresh cycles for patients with stage 3, 4 endometriosis as compared with cumulative pregnancy and live born rate of fresh cycles with stage 1, or 2 endometriosis, and tubal cause of infertility.
[This table is from Kuivasaari et al. (2005)]

| Cause of infertility | Cumulative pregnancy rate | Cumulative live birth rate |
|---|---|---|
| Endometriosis stage 1 , 2 | 64.5% | 51.6% |
| Endometriosis stage 3, 4 | 44.8% | 32.8% |
| Tubal infertility | 69% | 40.2% |

**Table 2.34(b)** Cumulative pregnancy and live- born rates for cycles with endometriosis when frozen embryo transfer was included.
[This table is from Kuivasaari et al. (2005)]

| Cause of infertility | Cumulative pregnancy rate | Cumulative live birth rate |
|---|---|---|
| Endometriosis stage 1, 2 | 67.7 % | 55.8% |
| Endometriosis stage 3, 4 | 56.7 % | 40.3% |
| Tubal infertility | 81.6 % | 43.7% |

In our study, we found that the outcome of IVF-ET is affected by the cause of infertility with highest pregnancy and implantation rate were when there is tubal, unexplained and PCOS (61.9%, 37.55%), (46.4%, 29.62%) and (41.7%, 27.5%) respectively; see table 2.24(a), figure (2.18) but we found that the distribution of low quality embryos "embryos with grade 2 and 3" was mainly among cycles in which there was endometriosis see table 2.24(b). This is probably the reasons behind the low outcome of cycles involving endometriosis.

Barnhart et al. (2002) found that the chance of achieving pregnancy was significantly lower for endometriosis patients [OR 0.56; 95% CI (0.44-0.70)], when compared with tubal factor controls. Multivariate analysis also demonstrated a decrease in fertilization and implantation rates, and a significant decrease in the number of oocytes retrieved for endometriosis patients. Pregnancy rates for women with severe endometriosis were significantly lower than for women with mild disease [OR 0.60; 95% CI (0.42-0.87)]. They concluded that patients with endometriosis undergoing IVF respond with significantly decreased levels of all markers of reproductive process, resulting in a pregnancy rate that is almost one half that of women with other indications for IVF. In addition, they suggested that the effect of endometriosis is not exclusively on the receptivity of the endometrium but also on the development of the oocyte and embryo.

Akande et al. (2004) found that women with minimal endometriosis have a lower probability of pregnancy compared with women with unexplained infertility 36% versus 55%; P <0.05. In addition, they found that other factors like primary infertility, smoking and longer duration (>3 years) of infertility were adversely associated with

pregnancy in women with unexplained infertility whilst the relationship was more complex in women with minor endometriosis.

### 2.6.8.3     *Ectopic Pregnancy:*

From the analysis of the cause of infertility, we notice that patients with a history of ectopic pregnancy and patients with hydrosalpinges had a low chance of becoming pregnant after IVF-ET as compared to tubal cause. The underlying cause for that is not known for ectopic pregnancy. Research by Lesny and co-workers found that difficult embryo transfers are associated with a greater risk of ectopic pregnancy, and they reported a case of ectopic pregnancy after TMET (Lesny et al. 1999b). This may explain the association of a hyperactive junctional zone with ectopic pregnancy and as we know from Fanchin et al the detrimental effect of junctional zone contraction on the outcome of IVF-ET.

### 2.6.9 The relationship between the cause of infertility and the grade of embryos:

In our study, we saw that the highest proportion of low grade embryos (grade 2,3) was among the cycles in which the cause of infertility was endometriosis (22%) and male factor infertility (16.6%), while the highest proportion of high grade embryos (grade 4,5) was among cycles in which the cause of infertility was PCOS. (P=0.051). In the case of endometriosis, there may be other underlying causes of low pregnancy rate rather than embryo quality, such as junctional zone contraction, and similar ideas can be applied to the cases of patients with a previous history of ectopic pregnancy.

In case of hydrosalpinx, the underlying cause may be due to a permanent defect in the endometrial receptivity resulting from the toxic effects of the fluid drained from the hydrosalpinx to the endometrium and not due to low grade embryos. In our study we found that the presence of a hydrosalpinx does not impair the quality of embryos transferred but seems to impair the implantation process. This may be due to leakage of fluid into the uterine cavity, which may disturb the receptivity of the endometrium and/or the developing embryos.

## 2.7 Conclusion:

Many factors can affect the outcome of an IVF-ET cycle. These factors include female age being less than 36, the quality of embryos, day of embryo transfer and the type of embryo transfer procedure. The most important parameters relating to the embryo are the grade of the embryo and blastomeres count. We found that when the grade of embryos is 4 or 5, or when the blastomeres count is 4 at day 2, and 8 at day 3, the chance of implantation is around 40%; see tables (2.14) and 2.18(a),(b); figures (2.11), (2.12) and (2.13). This result supports the idea of transferring only one embryo in order to avoid the risk of multiple pregnancy, by a proper selection of the embryo in a patient with good prognosis to avoid a low pregnancy rate. The potential low pregnancy rate can be compensated with the transfer of one blastocyst. This may lead to improved outcomes but further assessment requires a randomised study. For patients above 36 and those with unfavourable prognosis it would be better to continue double embryo transfer in order to avoid low pregnancy rates.

Although the pregnancy and the implantation rate for frozen embryos is lower than for fresh embryos, cryopreservation of the good quality embryos should also be considered in order to make future IVF cycles cheaper and less stressful physically for the female with one egg retrieval, patient can have many embryo transfer with out exposure to the risk of hyperstimulation.

We found also that there is a statistically significant difference in the outcome of day 2 and day 3 embryo transfers, with better outcome with day 3 ET, see table (2.20 ), figure (2.14) which means that we should consider delaying embryo transfer one day as that would give us more time for selection of good quality embryos.

In case of TCET there is no statistically significant difference in the outcome of embryo transfer when the procedure is easy or difficult; see table 2.21(b), figure (2.15). This supports the results of several published studies (Wood et al. 1985; Egan et al. 1990; and Tur- Kaspa et al. 1998). However in case of TMET, in our study we found that the outcome of the TMET is low although the difference is not statistically

significant may be due to the fact that we had only a few cases. This low outcome may be explained by the exaggeration of the junctional zone contraction with its negative effect on the outcome of the IVF-ET. Because of this ,TMET should not be done as a routine and should be done only in rare cases when the Mock ET is impossible or very difficult due to cervical atresia, stenosis, or in the presence of false passage. In such cases if the patient has at least one patent fallopian tube, GIFT or ZIFT should be considered because the success rate is higher and there will be no disturbance of the endometrium by the transfer catheter. In our study there was one patient who had a false passage in the cervical canal, which was confirmed by hysteroscopy. The patient went on to have 2 negative TMET cycles followed by a third cycle with ZIFT from which the result was positive and at the time of writing this thesis she was 9 weeks pregnant and foetal heart and foetal movement positive.

The cause of infertility is a very important parameter. Previous history of ectopic pregnancy, hydrosalpinx, endometriosis or high FSH have a potential negative influence on the outcome of the embryo transfer; while tubal, PCOS and unexplained factors have result in a much more positive chance as regards the outcome of IVF-ET.

In many IVF units nurses are the professional group with whom couples have most contact during treatment; they play a fundamental role in caring for infertile couples. In our study, we found that the outcome is good when a nurse do the embryo transfer procedure this will encourage to leave all the straightforward procedures to be done by the nurse.

# CHAPTER THREE

# *Differences in the Outcome of IVF-ET between Egg Donors and Recipients in an Egg Sharing Program*

## *3.0 Introduction:*

The data analysed in chapter 2 has demonstrated that one of the main negative effects on IVF/ET outcome is female age and that this effect is due, at least in part, to a lowering of embryo quality with advancing female age. However, it was not possible to determine from these data if there is also a component of the age effect that is due to endometrial factors.

## *3.1 Aim of the study:*

The aim of this study was to assess whether ageing of the ovary or ageing of the uterus has the greater negative effect on the outcome of IVF-ET.

## *3.2 Patients:*

This retrospective study included 31 couples who were treated in the egg sharing program of Hull IVF unit from 1st January 2000 to 31st Dec 2004. It also included all recipients who received their eggs from altruistic donors in the period from January 1993 to 31 of December 1999, after this date the program of altruistic donors was not stopped but there was no altruistic donors, the reason was unknown, and all the recipients were receiving their eggs from egg sharing donors.

## 3.3 Design and setting:

This was a retrospective study in the setting of a single academic research centre based at the Hull IVF unit. The unit has been providing IVF treatment for 20 years and oocyte donation for the last 14 years.

## 3.4 Method:

Among 148 cycles which were included in this retrospective study, there were 31 cycles of egg sharing patients, the corresponding 31 cycles of egg sharers in the egg sharing program, and the remaining 86 cycles were egg recipients (i.e. they received their eggs from altruistic donors). The total number of cycles of egg recipients (i.e. egg sharers plus recipients from altruistic donors) were collected together to show the outcome in different age groups of recipients, in order to determine whether aging of the ovary or the uterus is more important. We tried to determine whether there is a detrimental effect of the supra physiological hormone concentrations on the outcome of IVF treatment during egg sharing. More over we compared the outcome of standard IVF with the outcome of egg sharing in order to find whether there is any detrimental effect of egg sharing when the patient donates half of her eggs to other patients.

The protocol for oocyte donation required a detailed medical and family history to be taken from the donors, and all underwent hormonal assays for FSH, screening test for HBsAg, HIV, HCV, CMV and Chlamydia. In addition they had a chromosomal analysis and a screening test for the commonest genetic mutation of cystic fibrosis. Information regarding the donor's physical characteristics, medical history, educational background, occupation and interests was recorded. Matching of a donor with a recipient was based on physical characteristics.

All donor patients, whether in the egg sharing programme or altruistic donors, had the same long protocol of pituitary down regulation as standard IVF patients (for full details see chapter 2), with buserelin 1mg subcutaneously daily started at mid-luteal phase. Ovarian stimulation was carried out with hMG, with the starting dose depending on age between 150iu and 250iu daily. When the leading follicles reached a pre-ovulatory size (18–22mm), 10 000 IU of hCG was administrated. Oocytes were

aspirated using transvaginal ultrasound guidance 34–36 hrs after hCG administration. In the egg sharing programme oocytes were shared with the recipients when the number of aspirated eggs equalled or exceed 8, otherwise all oocytes were retained by the sharer. Oocytes from altruistic donors were usually shared between two recipients. All embryos were allowed to cleave and the best two or three embryos were selected for transfer. Cryopreservation was offered to the recipient couple if more than two good quality embryos remained after transfer. Fresh embryo transfer was performed on day 2 or day 3. All recipients and egg-sharing patients started oral progesterone on the day of egg retrieval, and continued with vaginal administration after embryo transfer until a pregnancy test was performed 2 weeks after embryo transfer.

The hormone replacement protocol for recipients depended on whether they had any ovarian function or not. For patients with ovarian function a long protocol with buserelin for down regulation was commenced simultaneously with the donor. A transvaginal ultrasound scan was performed on day 3 or 4 to check the endometrial thickness and the ovarian activity. If the endometrial thickness was <5 mm and no ovarian cysts were seen, patients commenced 100 µg oestradiol patches (Evorel Janssen-Cilag Ltd, UK) at a starting dose of one oestradiol patch on alternative days. The dose was then adjusted according to the endometrial thickness, aiming to achieve a double thickness of 10mm. 10 mg of oestradiol valerate orally daily and progesterone (Utrogestan 100mg Laboratories Besins International Paris France) 6 tablets at bed time orally was started 4 days before embryo transfer. For women with no ovarian function, oestradiol patches were started when the egg-sharer commenced ovarian stimulation. All recipients were started on a progesterone supplement, Utrogestan, from 4 days before embryo transfer, and continued until a pregnancy test was performed.

Data were analysed by Statistics Package for Social Sciences (SPSS, Surrey, UK). The outcomes recorded from the notes were pregnancy rate, implantation rate, abortion rate and live birth rate.

## 3.5 Results:

In the study, there were 31 cycles in which patients were seeking treatment for infertility but they were also donors, sharing their eggs with other patients. The

recipient either shared eggs with the donor in an egg sharing program or received eggs from a fertile woman who had donated their eggs altruistically, for the sake of helping other people. The cause of infertility of the recipient varied as follows: In 4 cycles the cause was idiopathic primary ovarian failure, in 1 cycle the patient had Turner's Syndrome, in 2 cycles both partners were carriers for cystic fibrosis, in 38 cycles the patients had previously responded poorly to stimulation, in 24 cycles the cause was poor eggs or poor embryos, in 46 cycles the cause was premature ovarian failure, in 1 cycle the patient had previous pelvic radiotherapy, in 1 cycle the patient had chemotherapy, see table 3.1, the grade of embryos in all donors and all egg sharers and all egg recipient cycles are shown in table 3.2.

Donors are always aged less than 35 years old in the UK because of the HFEA guidelines. Donors conceived successfully in 11 of the egg-sharing cycles and the pregnancy and implantation rates were 35.5% and 18.33% respectively. The recipient cycles were divided according to their age. In 42 cycles the recipient's age was less than 35. 16 of these cycles were positive and the pregnancy and implantation rates were 38.1% and 18.27% respectively. In 20 cycles, the recipient's age was between 35 and 39. 7 of these cycles were positive, pregnancy and implantation rates were 35% and 26.9% respectively. In 54 cycles the recipient's age was 40 or above. In 19 of these cycles the results were positive and the pregnancy and implantation rates were 35.18% and 15.96% respectively, [P = 0.991 (> 0.05), Odds ratio, and 95% CI 0.875(0.288-2.655)] for the pregnancy rate, and [P=0.637, Odds ratio, and 95% CI 0.996(0.431-2.306)] for the implantation rate, see table (3.3), and figure (3.1). This means that the difference in the outcome is not statistically significant and the outcome of the IVF procedure depends on the age of the ovary and not on the age of the uterus. when we divided the recipient cycles further according to age we saw that there were 7 cycles in which the age of patient is equal to or more than 45 years but still the outcome is good and the pregnancy rate is 28.6%, see table (3.3.).

**Table 3.1** Aetiology of infertility in recipients of donated oocytes. All recipients of egg sharing and recipients from altruistic donors are included

| Aetiology of infertility | No. (cycles) |
|---|---|
| Primary ovarian failure | 4 |
| Turner Syndrome | 1 cycle |
| Both partners were carriers for cystic fibrosis | 2 |
| Poor response in the previous IVF cycles | 38 |
| Poor eggs or poor embryos in previous treatment cycles | 24 |
| Premature ovarian failure | 46 |
| Pelvic radiotherapy | 1 |
| Chemotherapy | 1 |

**Table 3.2** The grades of embryos obtained and transferred in all donors and recipients

| Grades of embryos transferred to egg sharers | n | Grades of embryos donated to recipients aged <35yrs | n | Grades of embryos donated to recipients aged 35-39yrs | n | Grades of embryos donated to recipients aged 39-44 yrs | n | Grades of embryos donated to recipients aged >45yrs | n |
|---|---|---|---|---|---|---|---|---|---|
| 2,3,3 | 1 | 4,4,5 | 1 | 3,3,4 | 2 | 4,4,4 | 1 | 4,4,5 | 1 |
| 2,2,3 | 1 | 3,4,5 | 1 | 2,3,3 | 1 | 3,4,4 | 1 | 3,3,4 | 1 |
| 4,5 | 1 | 3,3,4 | 1 | 4,5 | 2 | 3,3,4 | 2 | 3,4 | 1 |
| 4,4 | 3 | 3,3,3 | 1 | 4,4 | 2 | 3,3,3 | 1 | 3,3 | 2 |
| 3,4 | 9 | 2,3,3 | 1 | 3,4 | 2 | 2,3,4 | 2 | 2,3 | 2 |
| 3,3 | 11 | 2,2,3 | 3 | 3,3 | 3 | 2,2,3 | 2 | 2 frozen ET | 1 |
| 2,3 | 2 | 2,2,2 | 1 | 2,3 | 5 | 4,5 | 5 | | |
| 4 | 3 | 4,5 | 3 | 4 | 1 | 4,4 | 2 | | |
| | | 4,4 | 2 | | | 3,4 | 7 | | |
| | | 3,4 | 9 | | | 3,3 | 14 | | |
| | | 3,3 | 7 | | | 2,3 | 5 | | |
| | | 2,4 | 1 | | | 2 frozen ET | 2 | | |
| | | 2,3 | 5 | | | 1 frozen ET | 1 | | |
| | | 2 frozen ET | 5 | | | 1 ET grade 3 | 2 | | |
| | | 4 | 1 | | | | | | |

**Table 3.3** Difference in the outcome between egg sharers their age is always <35 years, and different age groups of recipient, the difference in the outcome was checked with Chi square, and logistic regression test

| | Donor cycles (all < 35) | Total recipients | Recipient age < 35 | Recipient age 35-39 | Recipient age ≥ 40 | P value Odds ratio and 95% CI | Recipient 45 yrs and above |
|---|---|---|---|---|---|---|---|
| Total | 31 | 117 | 42 | 20 | 54 | | 7 |
| Pregnancies | 11 | 42 | 16 | 7 | 19 | | 2 |
| Pregnancy rate/ET | 35.5% | 35.89% | 38.1% | 35% | 35.18% | P 0.991 95% CI 0.875 (0.288-2.655) | 28.6% |
| Implantation rate/Embryo | 18.33% | 18.06% | 18.27% | 26.9% | 15.96% | P 0.637 95% CI 0.996 (0.431-2.306) | |
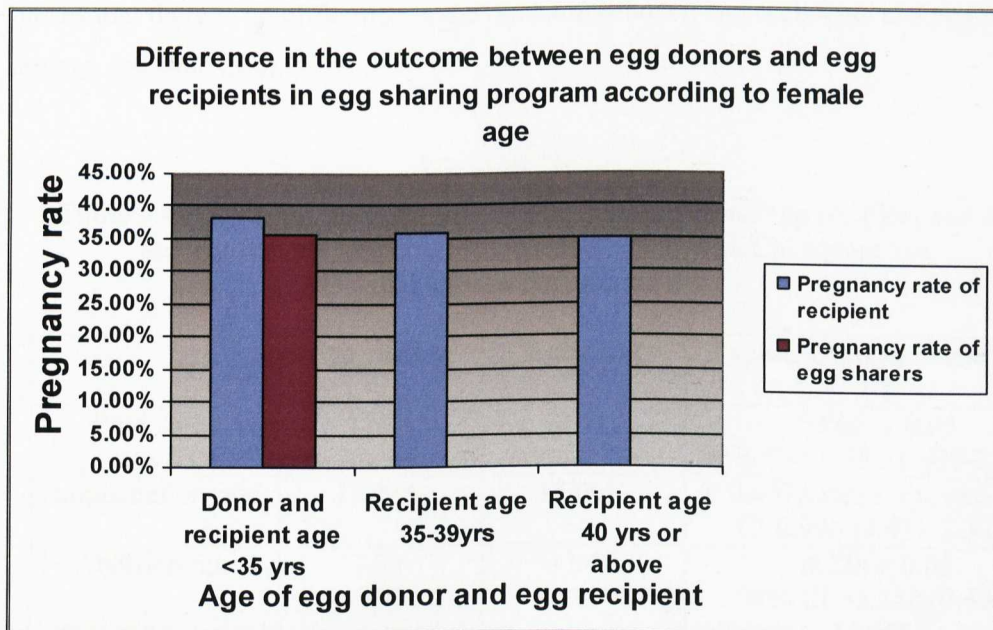


**Figure 3.1** Difference in the pregnancy rate between egg sharers and egg recipients in the egg sharing program. The outcome of egg donors was compared with the outcome of egg recipient of the same age because all donors are below 35 years; we found that there is no statistically significant difference regardless of the age group of the recipient.

In our study, we found that for egg sharing there were 11 positive results from 31 cycles and pregnancy rate per embryo transfer was 35.5%. In total for the recipients 42 out of 117 cycles were positive and the pregnancy rate 35.89% [P 0.966 > 0.05, 95% CI= 0.982(0.430 -2.246)], and the implantation rate for the total egg sharers versus egg recipients were 18.33%, and 18.06% respectively[P 0.637, 95% CI 0.996 (0.431-2.306)], see figures (3.1), and (3.2) . Among the egg sharer positive cycles there was one abortion and the abortion rate was 9%. The live birth rate for the egg sharers was 32.25%. In the recipient positive cycles there were 11 abortions and the abortion rate was 26.19% with live birth rate was 26.5% [P=0.228 > 0.05, Odds ratio, and 95% CI =3.548 (0.406-31.005)] for the abortion rate, and [P=0.524 > 0.05, Odds ratio, and 95% CI = 1.321 (0.560 – 3.115)] for live birth rate, see table (3.4) and figure (3.3). We can see that the pregnancy rate for the recipient < 35 years old (38.1%) is almost the same as the pregnancy rate of egg sharers (35.5%), and the abortion rate is higher and consequently live birth rate were lower in the total recipient cycles as compared to egg sharing cycles, but the difference did not reach to statistically significant level, which means that there is no difference in the outcome between egg recipients and egg sharing donors, see table (3.4).

**Table 3.4** Pregnancy, abortion, and live birth rates of total egg recipient and egg sharing donors. The difference were checked with Chi square test, and logistic regression tests

|  | Egg sharing | Recipient | P value, Odds ratio and 95% CI |
|---|---|---|---|
| Pregnancy rate/ET | 11/31 35.5% | 42/117 35.89% | 0.966 > 0.05 95% CI= 0.982(0.430-2.246) |
| Implantation rate | 18.33% | 18.06% | P 0.637,Odds ratio, and 95% CI 0.996 (0.431-2.306) |
| Abortion rate | 1/11 9% | 11/42 26.19% | 0.228 > 0.05 95% CI =3.548 (0.406-31.005) |
| Live birth rate / ET | 32.25% | 26.5% | 0.524 > 0.05 95% CI = 1.321 (0.560 – 3.115) |

## Difference in the implantation rate between egg sharers and egg recipients
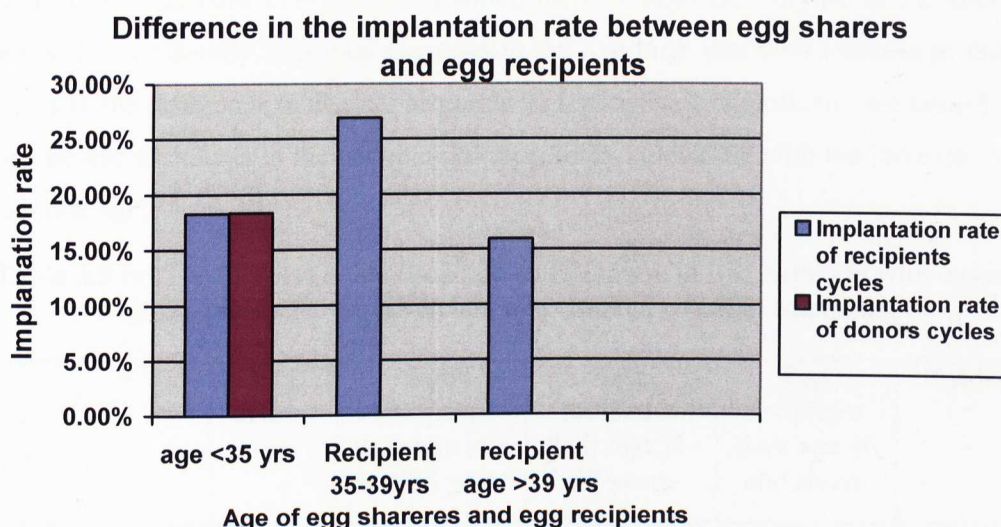


**Figure 3.2** Difference in the implantation rate between egg sharer and egg recipient. The difference is statistically not significant

## Difference in abortion and live birth rate between egg sharers and egg recipients
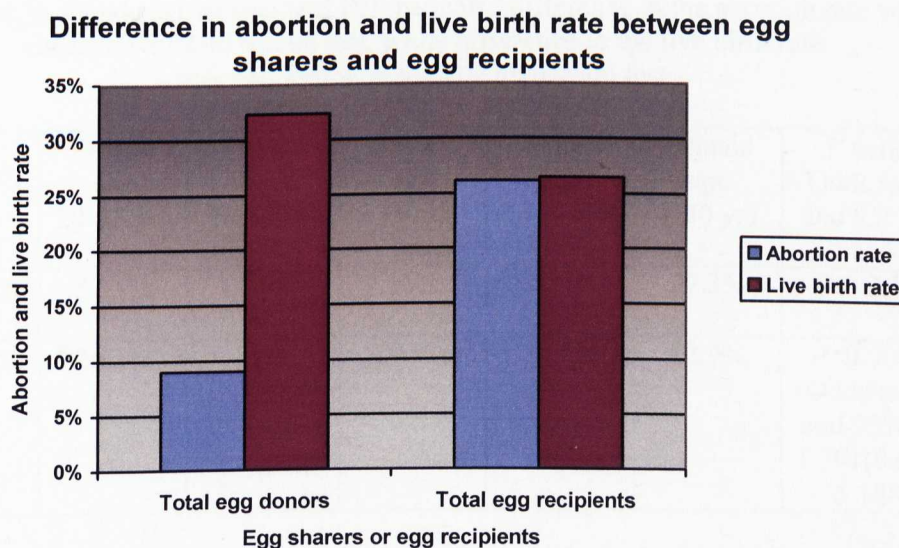


**Figure 3.3** Difference in the abortion and live birth rate between total egg sharers cycles and total recipients cycles. There is high abortion rate and lower live birth rate in recipients cycles as compared to total egg sharers cycles

Moreover, if we choose only recipient cycles, although the number is small, we found that there is stepwise increase in the abortion rate and stepwise decrease in the live birth rate with increase in recipient age, $P>0.05$, see table 3.5(a) same result was

found in the standard IVF cycles, in which there is stepwise increase in the abortion rate and consequently step wise decrease in the live birth rate with increase in female age, and the difference in the live birth rate was statistically significant, see table 3.5 (b) may be due to defects in the endometrial receptivity coinciding with the increase in the maternal age.

**Table 3.5 (a)** The increase in abortion rate and decrease in live birth rate with increase in recipient age, the difference was checked with Chi square test

|  | Egg donors | Egg recipients their age less than 35 years | Egg recipients their age 35-39 years | Egg recipients their age 40 and above | P value |
|---|---|---|---|---|---|
| Abortion rate | 9% | 18.75% | 28.57% | 31.56% | 0.606 >0.05 |
| Live birth rate | 32.25% | 30.95% | 26.31% | 23.21% | 0.771 >0.05 |

**Table 3.5(b)** Increase in The abortion rate and decrease in the live birth rate with increase in female age of standard IVF patients, difference in the abortion rate was checked with Chi square test, while difference in the live birth rate was checked with logistic regression test

|  | Female age 20-25 yrs | Female age 26-30 yrs | Female age 31- 35 yrs | Female age 36 –39yrs | Female age ≥ 40 yrs | P value, Odds ratio, and 95% CI |
|---|---|---|---|---|---|---|
| Abortion rate | 37.5% | 12.34% | 16% | 22.7% | 27.3% | P = 0.237 |
| Live birth rate | 26.3% | 39% | 29.9% | 20.5% | 12.9% | P<0.0001, Odds ratio, and 95% CI 1.791(0.618-5.188) |

In addition, if we compare the pregnancy rate of recipient's cycles with ovarian function, with the pregnancy rate of recipient's cycles with no ovarian function, we found that there were 63 cycles in which there is ovarian function, out of these, 26 cycles were positive, making the pregnancy rate 41.3%. Further more we had 54 cycles in which there is no ovarian function, out of these, 16 cycles were positive, and the pregnancy rate was 29.62%, P 0.216 (>0.05), see table (3.6). Although the difference did not reach to a statistically significant level, we found that the outcome is better

when there is ovarian function, the difference may become significant with higher number of cases, and the explanation for this result may be due to defect in the endometrial function probably resulted from long time exposure of the endometrium to low oestrogen level when there is ovarian failure.

**Table 3.6** Difference in the outcome between recipients with ovarian function and recipients with no ovarian function

|  | Recipients with ovarian function | Recipients with no ovarian function | P value |
|---|---|---|---|
| Pregnancy rate | 41.3% | 29.62% | 0.216 >0.05 |

Moreover, if we compared the outcome of egg sharing patients with the outcome of standard IVF patients of Hull IVF unit who had the same stimulation treatment, had the transfer of same quality embryos, and the embryo transfers were done by the same operators (see chapter two) there are 771 cycles of IVF-ET and IVF/ICSI-ET cycles in which the female age was less than 35 years old and had 2 embryo transfer. 314 cycles were positive making the pregnancy and the implantation rate 40.7% and 25.61% respectively. We found that egg sharing patients have lower pregnancy and implantation rate while the live birth rate of the egg sharers is almost the same of the standard IVF patients, see table (3.7) and figure (3.4). The low abortion rate for egg sharer, which is only 9%, may be due to the total number of egg sharer is small which is only 31, with higher number of egg sharers this rate may become more, and may be equal to the abortion rate of the standard IVF patients. From this result we can see that egg sharing patient have good outcome as compared to the standard IVF patients, and we can conclude that egg sharing program have no detrimental effect on the outcome of egg sharing patients , see table (3.7), and figure (3.4).

**Table 3.7** Difference in the pregnancy, implantation, abortion, and live birth rate between egg sharing cycles and standard IVF cycles

|  | Pregnancy rate/ET | Implantation rate/ET | Abortion rate | Live birth rate |
|---|---|---|---|---|
| Standard IVF-ET patients | 40.7% | 25.61% | 20.46% | 32.29% |
| Egg sharers patients | 35.5% | 18.3% | 9% | 32.25% |
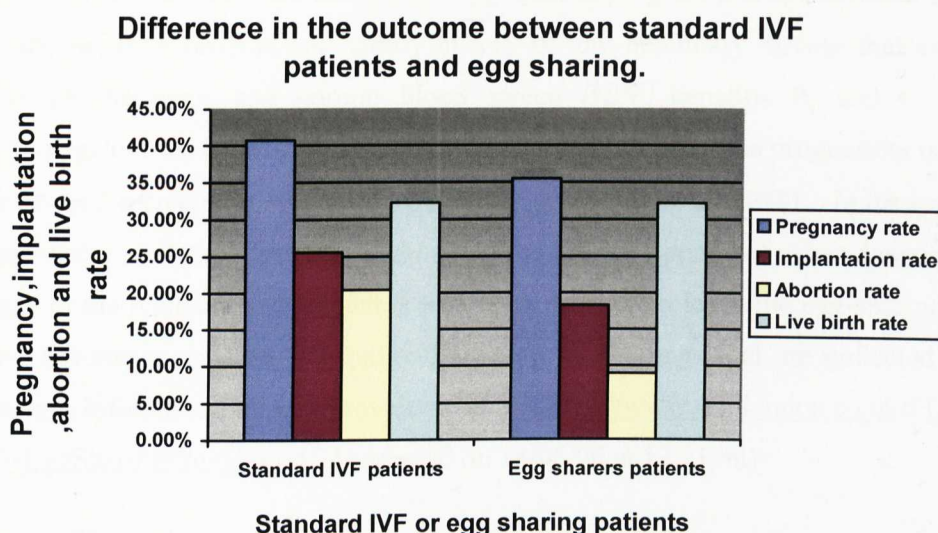
**Figure3.4** Difference in the pregnancy, implantation, abortion, and live birth rates between egg sharers and standard IVF patients. It shows that pregnancy and implantation rate is lower, live birth rate is higher, and implantation and abortion rates is lower in egg sharers as compared to the standard IVF patients.

# 3.6 Discussion:

## 3.6.1 Egg sharing programs:

The first successful pregnancy after oocyte donation was reported by Lutjen et al. (1984), and since then egg donation has proved to be a valuable tool in allowing couples who would otherwise have been unable to conceive to have children. Currently in the UK there is a shortage of supply of donor eggs compounded by an increase in demand (Abdulla 1996; Kan et al. 1998). As many couples have difficulty in funding their own fertility treatment, couples sometimes agree to share any excess eggs retrieved with other women in exchange for a reduction in the cost of their own treatment. This process, known as egg sharing, which is an effective way to provide treatment for patients who need help funding their own fertility treatment as well as providing eggs for couples who require them. Paid egg donation is not permitted in the UK (Johnson1997).

The criteria to be a donor in an egg-sharing program are: age between 21 and 35 years, negative medical and family history of any hereditary disease that could pass through the eggs, and normal blood screen (HIV, hepatitis B, and C, syphilis, cytomegalovirus, cystic fibrosis and karyotype). The aim of the programme is for each sharer and recipient to receive at least four eggs (Ahuja et al. 1998). In the event of an egg-sharer producing less than eight eggs, she has the options of either donating all the eggs to the recipient and to have a free cycle later or to leave the egg-sharing scheme and become a standard IVF patient, keeping all the eggs that are collected with no charge (Information for egg providers 2002) ©http://www.crmlondon.co.uk/PDFfiles/I-13-EggSharingProvider.pdf (Accessed on 14/05/06 at 12.11pm)

The donors are free to withdraw from the egg-sharing scheme at any time before the recipient has had her embryo transfer (Information for egg providers 2002). The donation is performed anonymously and no information will ever be given out to any individual or parties other than the Human Fertilisation & Embryology Authority (HFEA). On 1st of April 2005 a new law came into effect, which allows people conceived through donation to find out who the donor was once they reach the age of 18 years (The National Gamete Donation Trust) http://www.ngdt.co.uk/( Accessed on 2/07/06 at 11.12am).

In an egg-sharing program, it is important not to ignore the psychological and ethical aspects of such a treatment. It has been suggested that it could be emotionally traumatic for an egg-sharer, desperate for a child, to consider that although her treatment has been unsuccessful, recipients could be pregnant with her eggs and therefore all egg-sharers and recipients attend counselling sessions prior to embarking on treatment. Counselling is also available during and after treatment as patients may also experience an extra psychological burden. In a 1997 British survey, it was found that 8% of 79 donors who failed to become pregnant reported experiencing such distress (Ahuja et al. 1998).

Kan et al. (1998) performed a questionnaire based study of 501 women enquiring about anonymous oocyte donation at a private IVF clinic and they investigated the demographic characteristics and logistical issues involved in ovum donation. They found that women in full-time employment were the majority of actual

donors, and logistical factors, such as the distance to travel, time involved and social commitments during the stimulation period, as well as concerns about complications were major reasons for non-donation (Kan et al. 1998).

Several studies have looked at the difference in the outcome between egg-sharers and recipients (Check et al. 1992; 1995; 1999; 2001; and Ahuja et al. 1996).

A study by Check and co-workers in 1992 found that there is a statistically significant difference in the pregnancy rates per retrieval (10.5% donors versus 29% recipients), and per transfer (11.1% versus 32.3%). In addition, they found that abortion rates were similar (25% donor, 27.2% recipients). The authors suggested that the underlying cause for this lower outcome in the donor when compared to the recipient is possibly due to a negative effect of hyperstimulation or to the adverse endometrial environment of the donor (Check et al. 1992). Similar results were seen in another study (Ahuja et al. 1996). This group found that there were more births per patient amongst recipients than amongst donors (30% versus 20%). The authors therefore concluded that an egg-sharing program is a very constructive way of solving the problem of the shortage of eggs for donation. In our study we found that the pregnancy and implantation rates of egg sharers in which female age is <35 years old as compared to egg sharing donors is almost the same 38.1% versus 35.5% respectively, and 18.27% versus 18.33 respectively.

Check et al. (1995) found that there is a difference in the outcome between recipients with ovarian function and other recipients with no ovarian function following both Fresh and frozen embryo transfers, and they found that clinical pregnancy and implantation rates per transfer for fresh embryo transfers were 17.5% and 7.5% respectively for donors, 20.4% and 8.6% respectively for recipients with ovarian function, and 46.3%, 15.6% for recipients with ovarian failure (P < 0.05), see table 3.8(a), (b). The corresponding pregnancy and implantation rates for frozen embryo transfers were 15.3% and 5.1% for donors, 17.2% and 5.2% for recipients with ovarian function and 23.8% and 7.1% for recipients with ovarian failure, see tables 3.8(a), and 3.8(b), in addition, they found that there is a difference in the clinical pregnancy rate between recipients with ovarian function and their age is equal or more than 40 and recipients with ovarian function but their age is below 40, and the pregnancy rate was

14% versus 22.2% respectively, same result was found for recipients with ovarian failure 33.3% for the older group of women and 39.4% for the younger group. Furthermore, they found that ovarian function was the only factor to have an independent effect on outcome (Check et al. 1995), see table 3.8(c).

**Table 3.8(a)** Difference in the pregnancy rate between egg sharers and two groups of recipients one with ovarian function and one with ovarian failure in fresh and frozen cycles. [This table is from Check et al. (1995)]

|  | Clinical PR for donor | Recipient with ovarian function | Recipient with no ovarian function | P |
|---|---|---|---|---|
| Fresh cycles | 17.5% | 20.4% | 46.3% | < 0.05 |
| Frozen cycles | 15.3% | 17.2% | 23.8% | > 0.05 |

**Table 3.8 (b)** Difference in the implantation rates between egg sharers and the two groups of recipients one with ovarian function and one with ovarian failure in fresh and frozen cycles. [This table is from Check et al. (1995)]

|  | Implantation rate for donors | Recipient with ovarian function | Recipient with no ovarian function | P |
|---|---|---|---|---|
| Fresh cycles | 7.5% | 8.6% | 15.6% | < 0.05 |
| Frozen cycles | 5.1% | 5.2% | 7.1% | > 0.05 |

**Table 3.8(c)** Difference in the pregnancy rate between recipients with ovarian function aged ≤ 40 and >40 and recipients with ovarian failure aged ≤ 40 and > 40. [This table is from Check et al. (1995)]

|  | Recipients with ovarian function aged < 40 | Recipients with ovarian function aged ≥ 40 | Recipient with ovarian failure aged < 40 | Recipients with ovarian failure aged ≥40 |
|---|---|---|---|---|
| Pregnancy rate | 22.2% | 14% | 39.4% | 33.3% |

Check et al. (1999) found a higher implantation rate (39% versus 22.5%; P < 0.05) in recipients compared to donors in stimulated cycles, and no differences were seen in the pregnancy or implantation rates in frozen ET cycles. They concluded that superior implantation rates and pregnancy rates in oocyte recipients versus donors were

related to the hyperstimulation regimen rather than to better oocyte quality for recipients because of egg sharing or to a better uterine environment because of similar results with frozen ET in all three groups (Check et al. 1999).

A study done by the Society for Assisted Reproductive Technology and the Centres for Disease Control and Prevention for the year 1995 (Ovarian Aging and Infertility) http://www.havingbabies.com/age-infertility-ovarian.html (Accessed on 19/08/06 at 5.34 pm), They compared the outcome of IVF-ET for a group of recipients when they used their own eggs and the outcome of another group after using eggs from donors and found that live birth rates per transfer remain the same when donor eggs were used while using their own eggs live birth rate became lower at the age of 35 years and further decreased with increases in maternal age, see figure (3.5).
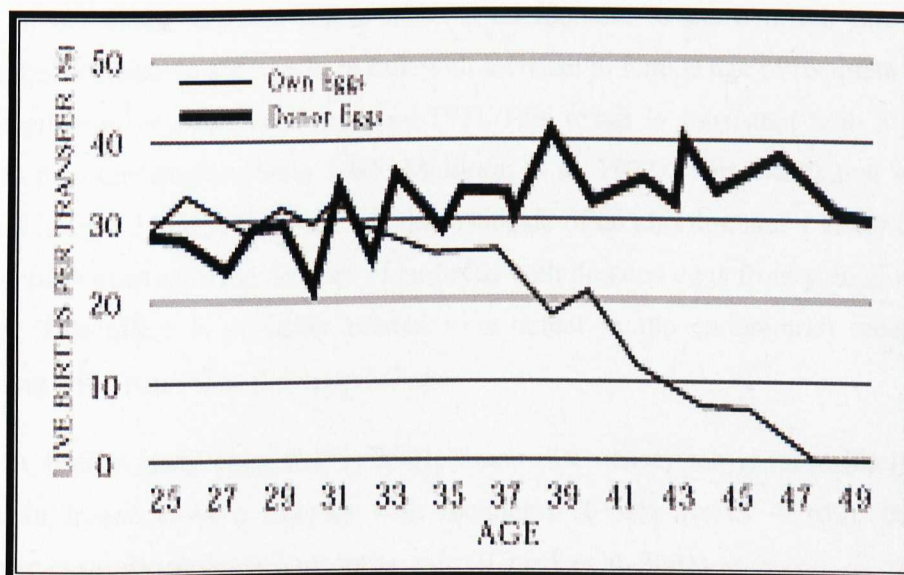


**Figure 3.5** Live births per transfer of fresh embryos from own and donor eggs, by age of recipient.
This figure is Adapted from SART/CDQ report for ART clinics, 1995, December, 1997. (Ovarian Aging and Infertility)© http://www.havingbabies.com/age-infertility-ovarian.html (Accessed on 19/8/2006 at 5.34pm).

Advancing age in women is associated with an accelerating impairment in the ability to conceive and an increasing risk of miscarriage or of chromosomal abnormality in surviving offspring. These effects can be attributed to defective oocyte quality, as indicated by the success of oocyte donation treatment (Balmaceda et al. 1994). There is some evidence of a decrease in the functional capacity of the human uterus in older reproductive age women with an age-related increase in the number of spontaneous abortions in which the embryo is apparently chromosomally normal (Stein 1985).

Meldrum et al. (1992) found that significantly fewer ongoing and delivered pregnancies occurred in recipients aged >40 years receiving oocyte donation from young women than in a similar group of younger recipients (8% versus 43%, P<0.02). A significant improvement was achieved in another group of women over 40, treated with a doubled dose of progesterone, (46% ongoing and delivered, P<0.01), and they suggest an effect of ageing on uterine receptivity that is at least partly correctable by progesterone stimulation of the endometrium.

In our study, we found that there is an increase in the abortion rate and a consequent decrease in the live birth rate with increase in female age of recipient in egg sharing program or patients of standard IVF. This result is consistent with a results found in previous studies (Stein 1985; Meldrum et al. 1992). This means that when a recipient is aged 35 and above she has more chance of an abortion and a lower chance of a live birth even after the transfer of embryos with donated eggs from young, normal females. This effect is probably related to a defect in the endometrial receptivity coinciding with increase in the maternal age.

A further study published in 2001, found that conception is more likely after fresh than frozen embryo transfer with recipients (63.4% versus 43.6%). but this difference is similar to donor conception rates (Check et al. 2001).

Thum et al. (2003) found that the average amount of gonadotrophin required to achieve follicular maturity, the average number of oocytes collected and the chance of achieving a pregnancy or live birth were not significantly different between egg-sharers and standard IVF/ICSI patients. They concluded that egg sharers are not exposed to a higher risk of ovarian hyperstimulation syndrome, and egg-sharing does not

compromise the outcome for an egg-sharer or a recipient as compared to standard IVF/ICSI patients. There was also no imbalance of egg allocation (Thum et al. 2003).

In our study, we found that the pregnancy, implantation, abortion and live birth rates for egg-sharer compared with standard IVF-ET patients (40.2% versus 35.5%), (25.61% versus 18.3%), (9% versus 20.46%) and (32.25% versus 32.29%%) respectively, see table (3.7) This means that egg sharing has no detrimental effect on the outcome of IVF-ET to egg donors patients.

# 3.7 Conclusions:

Egg sharing is a good treatment option for older women, for those who are carriers for hereditary disease and for women with ovarian failure. The most imperative conclusion is that participating in an egg-sharing program does not compromise the chance of achieving a pregnancy or live birth for the egg-sharer or the recipient compared with standard IVF/ICSI patients. Those patients participating in an egg-sharing programme are providing a valuable resource of donor eggs while not compromising their own treatment outcome or putting themselves at any additional risk of complications. In our study we found that pregnancy rates for the recipients were almost the same regardless their age, see table (3.3), and figure (3.1), therefore being the recipient of egg sharing is more attractive to older women. This method of egg donation will also help to alleviate the financial problems many couples face when they have to finance their IVF treatment themselves. However, the ethical and psychological aspects should not be ignored, especially as it has been suggested that it could be emotionally traumatic for a childless donor to consider that although her treatment has been unsuccessful, recipients could be pregnant with her eggs.

The only parameter which is alterable and which might affect success rates is the technique of ET itself.

*The next chapter therefore will discuss the choice of technique from published literature*

# CHAPTER FOUR

# A Review of the Literature Concerning the Technique of Embryo Transfer

## 4.1   Basic technique of embryo transfer:

Conventional IVF involves several related procedures, including ovarian stimulation, egg retrieval (Fleming et al. 1994; Filicori et al. 1999), semen preparation to select progressively motile spermatozoa (Drevius 1972; Pertoff et al. 1978), insemination of the eggs, in vitro culture, assessment of fertilization, assessment of cleavage (Staessen et al. 1989, 1992 and 1993), transfer of two or three embryos (Edwards and Brody 1995) and cryopreservation of excess embryos (Friedler et al. 1988).

Embryo transfer is the last and the most critical step in the IVF cycle but it has been changed very little since its introduction 1978 (Steptoe and Edwards 1978).
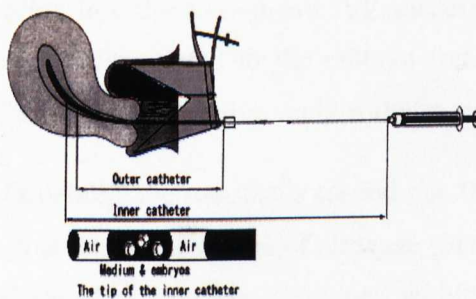


**Figure 4.1** Loading of the embryo transfer catheter and the technique of ET.
After insertion of the vaginal speculum the outer sheath of the transfer catheter is advanced to a point approaching, but not touching, the uterine fundus. The inner catheter loaded with the embryos is then inserted into the outer sheath. The transfer catheter contains 2 or 3 embryos in 2 drops of culture media and contained between 2 air bubbles.
Modified figure from Kojima et al. (2001)

In our retrospective study, see Chapter (2) we found a statistically significant difference in the pregnancy and implantation rates between day 2 and day 3 ET, with a

better outcome when embryo transfer procedure was delayed for one day, (i.e. day three instead of day two). This delay probably gives more time for selection of better quality embryos for transfer 35.1% versus 42.8% respectively (P<0.024), and 21.53% versus 27.43% respectively (P< 0.005), see table (2.20), figure (2.14). In addition, we found that a better outcome when the procedure was transcervical or transtubal if at least one tube was patent, while the outcome of transmyometrial embryo transfer (TMET) was low. We concluded that TMET should be done only in rare cases, and when the tubes are not patent. The difference between the pregnancy and implantation rates of easy versus difficult transcervical embryo transfer (TCET) were not statistically significant, 37.3% versus 40% respectively, P = 0.612 (>0.05), and 23.1% versus 26.31% respectively P = 0.242, and the pregnancy and implantation rates for TMET (both 10%), see table 2.21(b), figure (2.15).

Furthermore, our study showed a statistically significant difference in the outcome if the procedure done by any one of three different operators 35.2%, 41.7%, and 26.5% respectively (P<0.05), with better outcome when a nurse performed the procedure, see table (2.22), figure (2.15). In addition, we found that a new trainee would take around one year to become expert in doing the embryo transfer procedure, see table (2.23), and figure (2.17). We concluded from the previous analysis that embryo transfer is "the step in the IVF process most likely to be amenable to modification in order to improve IVF success rates". In this chapter, we will review published articles related to the embryo transfer procedure in order to compare the results from our retrospective study with the result obtained from other previous studies.

Generally, the transfer is carried out 48-72 hrs after oocyte collection when the embryo is at the 2-8 cell stage of cleavage (Staessen et al. 1992; 1993). Transfer is with a special catheter, typically positioned within the uterine cavity with its tip 5-15mm from the fundus (Lesny et al. 1998a; Mansour and Aboulghar 2002; Coroleu et al. 2002), see figure (4.1) and (4.2). In the UK the Human Fertilization and Embryology Authority (HFEA Act 1990) has ruled that a maximum of three embryos may be transferred although the majority of IVF centres transfer only two embryos in order to avoid the risk of a high order multiple gestation (HFEA Code of Practice 6th Edition).
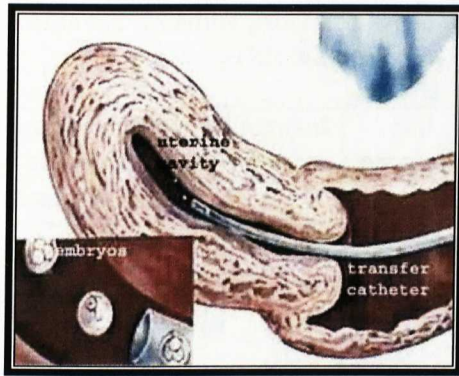
**Figure 4.2** Schematic illustration of the embryo transfer procedure. One or more embryos suspended in a drop of culture medium are drawn into a transfer catheter; the operator guides the tip of the loaded catheter through the cervix and deposits the fluid containing the embryos into the uterine cavity.
This figure is from
©Embryo transfer
http://www.drmalpani.com/book/chapter25c.html
(Accessed on 10/08/06, at 4:13pm)

## 4.1.1 Loading procedure of the embryo transfer catheter:

Studies have tried to optimise the embryo transfer technique. Poindexter et al. (1986) found that a large volume of the transfer media (>60 µL) and a large air interface may result in an increased risk of either embryo expulsion from the uterus, or of adherence to the outside of the catheter.

Meldrum et al. (1987) found that the pregnancy rate increased after reducing the volumes of both air and transfer media. Others found that increasing the viscosity of the transfer media did not increase the success rate (Menezo et al. 1989)

The liquid media containing the embryos is placed near the distal end of the catheter and contained between two air bubbles to avoid spillage of the embryos from the open end (Mansour and Aboulghar 2002). Evidence shows a positive correlation between culture media volume and the occurrence of an ectopic pregnancy (Marcus and Brinsden 1995).

Ebner and co workers found that loading the transfer catheter with a culture media volume of <10µl and including the air bubbles both had a negative effect on the implantation and pregnancy rates (Ebner et al. 2001), see table (4.1).

**Table 4.1** Loading process of the embryo transfer catheter and Outcome.
[This table is from Ebner et al. (2001)]

| Transfer related parameter | Pregnant | Not pregnant | Implantation Rate ( % ) | Pregnancy Rate ( % ) |
|---|---|---|---|---|
| Transfer volume | | | | |
| <10µL | 46 | 131 | 14.1 P <0.05 | 26  P <0.001 |
| 10-20µL | 59 | 68 | 19.1P <0.05 | 46.5  P <0.001 |
| Presence of air Bubbles | | | | |
| Yes | 17 | 90 | 9.3 <0.001 | 15.9 P <0.001 |
| No | 88 | 109 | 20.5 <0.001 | 44.7 P <0.001 |

The syringe used for embryo transfer catheter should be tested using a mouse embryo bioassay in order to confirm that it is not embryo toxic (Gardner and Schoolcraft 1999). The syringe should be pushed in a controlled way, so as not to push the embryo with such excessive force that they are either damaged or pushed in to the fallopian tube (Leong et al. 1986). To minimize the potential for movement and expulsion of the embryos following embryo transfer, a fibrin sealant "biological glue" has been used to try to attach embryos to the endometrium at the site of embryo deposition (Feichtinger et al. 1990). In a prospective, randomized study no significant difference in clinical pregnancy rate or ongoing pregnancy rate, but a significant reduction in ectopic pregnancies, was found by using this method (Feichtinger et al. 1992).

## 4.1.2 Routes and methods of embryo transfer:

The embryo transfer procedure has not been changed much since it was first described (Steptoe and Edwards 1978). It can be performed either into the fallopian tube or into the uterus.

### 4.1.2.1 Transcervical intrauterine placement of the embryo:

The majority of embryo transfers are currently performed by a non surgical approach. The transcervical method is a simple, rapid procedure with no analgesia required. However, the disadvantage of this method is the technical difficulty in patients

with cervical stenosis, which sometimes make this procedure very difficult, or even impossible (Wood et al. 1985; Sharif et al.1996; Noyes et al. 1999).

### *4.1.2.2 Transmyometrial intrauterine placement of the embryo:*

Kato et al. (1993) described another method of embryo transfer, transmyometrial embryo transfer (TMET). This procedure was performed for patients who had had very difficult embryo transfers in the previous cycle. The authors achieved a pregnancy rate of 36%. Another study performed by Sharif et al. (1996) used this method when a mock transcervical embryo transfer, performed immediately prior to actual embryo transfer, proved to be impossible, and achieved a pregnancy rate of 23%. The transmyometrial embryo transfer (TMET) as described by several groups (Kato et al. 1993; Sharif et al. 1996) is not difficult when performed under ultrasound guidance and does not require anaesthesia.

There is a case reported by Anttilla et al. (1999) in which a TMET was performed for an obstructive abnormality of the uterus. The patient had cervical atresia, diagnosed at the age of 24 years. Attempts to create a neocervix had been unsuccessful but since there was no sign of retrograde menstruation or of haematometra, with her consent a hysterectomy was not done. At the age of 32 years, a successful pregnancy was achieved after IVF and TMET, and she was delivered by emergency caesarean section at 32 weeks gestation due to sever pre eclampsia (Anttilla et al. 1999).

In another case reported by Asaad and Carver (1997) a patient with unexplained infertility received seven unsuccessful cycles of treatment over two and a half years, comprising four IVF-ET cycles using fresh embryos, one ET using frozen- thawed embryos and two attempts using fallopian sperm perfusion. In a final attempt, a new technique of embryo transfer was tried for her. Gestaldi et al. (1993) had previously described this technique. They described a technique of actual implantation of the embryo directly into the endometrium using a modified Monash catheter. Using this catheter four embryos were transferred and released at the junction of the endometrium and myometrium without puncturing the endometrium (the embryos were kept below the endometrium). The site of deposition was confirmed by ultrasound. The procedure was successful and the patient subsequently delivered twins (Asaad and Carver 1997).

Several studies have shown that difficult transcervical embryo transfer, including the use of a tenaculum, can stimulate junctional zone contractions (Lesny et al. 1998a; 1999a). One study by Biervliet et al. (2002) investigated the occurrence of junctional zone contractions after TMET. They found that after the insertion of a Towaco needle there was a significant increase in the number of random, opposing, and cervico fundal contractions (Biervliet et al. 2002). Junctional zone contractions have been shown to have a negative effect on the pregnancy rate (Fanchin et al. 1998b), and Lesny et al. (1999b) found that junctional zone contraction can dislocate the embryo from the uterine cavity and lead to an ectopic pregnancy. Based on the above results Biervliet et al. (2002) suggested the avoidance of TMET as a routine procedure, and recommended its use only when other options were impossible.

Kato suggested doing TMET for fragile embryos (Kato et al. 1993), and others have suggested employing it after multiple implantation failures (Asaad and Carver 1997; Groutz et al. 1997) or when the uterus is acutely anteverted, or when there is cervical stenosis (Sharif et al. 1996; Biervliet et al. 2002). An alternative strategy was described by the Bourne Hall group, They advised that carrying out a cervical dilatation after down regulation with an LHRH analogue prior to stimulation with gonadotrophin could result in a reduced incidence of difficult embryo transfers(Abusheikha et al. 1999).

### 4.1.2.3 *Zygote intrafallopian tube transfer (ZIFT):*

During this procedure, the fallopian tube can be cannulated either from the fimbrial end with the use of laparoscopy (orthograde), or through the uterine end with the guidance of ultrasound, with tactile sensation or with the use of hysteroscopy (retrograde) (Al Hussaini 1992). With this procedure, there is a risk of flushing the embryo from the tube if the injection flow rate is high (Woolcott and Stanger 1994). ZIFT can be undertaken only if the tube is patent and if the patient does not have a previous history of pelvic inflammatory disease (PID), otherwise there is a much increased risk of ectopic pregnancy (Al-Hussaini 1992). ZIFT may be indicated in cases of recurrent IVF failure (Al-Hussaini 1992).

## *4.1 3. When to transfer the embryos after insemination:*

### *4.1.3.1 Transfer of the embryos at the cleavage stage or at the blastocyst stage:*

At the time of ejaculation, 300 million sperm may enter the upper part of the vagina but only 1% (approx 3 million) enters the uterus. The sperm can survive for up to 48hrs in the genital tract. During IVF, each oocyte will be inseminated with about 100,000 sperm, and fertilization is not a single event but a continuum. It begins when the capacitated spermatozoa come into contact with the ovum, and the initial contact between them is a receptor mediated process (Shabonowitz and O Rand 1988). A number of glycoprotiens have been identified in the zona pallucida, termed ZP1, ZP2, and ZP3. Only ZP2 and ZP3 have been shown to possess biological functions. ZP3 is the most abundant and expressed only in a growing oocyte (Florman and Wassermann 1985; Bleil and Wassermann 1986). Sperm-zona binding is highly species specific (Saling and Storey 1979). Once the binding is accomplished, the acrosome reaction is triggered by the peptide chain component of the receptor glycoprotein (Shabonowitz and O Rand 1988).

After oocyte and sperm fusion, the oocyte will extrude the second polar body with its excess chromosomes. The fusion of the oocyte and the sperm nuclei by the formation of two pronuclei marks the creation of the zygote and the end of fertilization (Edwards et al. 1969).

At 1.5 to 3 days after ovulation, the zygote begins to cleave and each subsequent division increases the number of cells, which are termed blastomeres. Each division occurs approximately every 20 hrs (Cummins et al. 1986), and with each division the blastomeres within the zona pellucida become smaller and smaller. When the blastomeres number is 16-32 (day 3 to 4) the zygote is termed a morula (Edwards et al. 1980). At 4 to 5 days after fertilization, cell division continues and a cavity is formed at the centre. The cells will become more flattened while the zona pallucida remains the same size. At this stage, the embryo is called a blastocyst (Mohr and Trounson 1982; Howlett et al.1985).

The presence of the blastocyst indicates that two cell types are forming; the inner cell mass, and the trophectoderm (approximately 45 and 80 cells respectively) (Hardy et al. 1989). The inner cell mass then divides rapidly to form a two-layered disc. The top layer of cells forms the embryo and the amniotic cavity, while the lower cells become the yolk sac (Wiley 1987). Prior to implantation the blastocyst must break free from the zona pellucida, a process termed hatching. Around the 6th day after fertilization, the trophoblast cells secrete an enzyme that erodes the epithelial uterine lining and creates an implantation site for the blastocyst (Hardy et al. 1989).

The first clinical human pregnancy after in vitro fertilization was established by the transfer of a blastocyst (Steptoe and Edwards 1978). However, due to difficulties in maintaining the human embryo in culture for more than a couple of days, cleavage-stage transfers became routine. The recent introduction of sequential culture media, which can facilitate successful extended culture, has refocused attention upon the role of human blastocyst in IVF. The success of extended culture depends on many factors in the laboratory including medium composition (Gardner 2000).

In all mammalian species studied to date, the transfer of embryos to the uterus at the cleavage stage does not lead to as high a pregnancy rate as the transfer of morulas or blastocysts. This is true for both fresh embryo cycles (Schoolcraft and Gardner 2000) and frozen embryo cycles (Langley et al. 2001). In general, morula and blastocyst can tolerate a wider range of environments (Gardner et al. 2000). However, it is evident that human pregnancies can be established after asynchronous transfers (Ahuja et al. 1985). Like mammalian embryos, the human embryo has the ability to grow in a wide variety of culture conditions and it can adapt to its environment and continue its development (Gardner et al. 2000).

**Figure 4.3**
(a) Day 3 embryo 6-8 cells
(b) Day 5 embryo (blastocyst)
These figures from Victoria fertility centre ©http://www.victoriafertility.com/services/embryo-culture-transfer.htm (Accessed on 29/08/06 at 11.32 am)
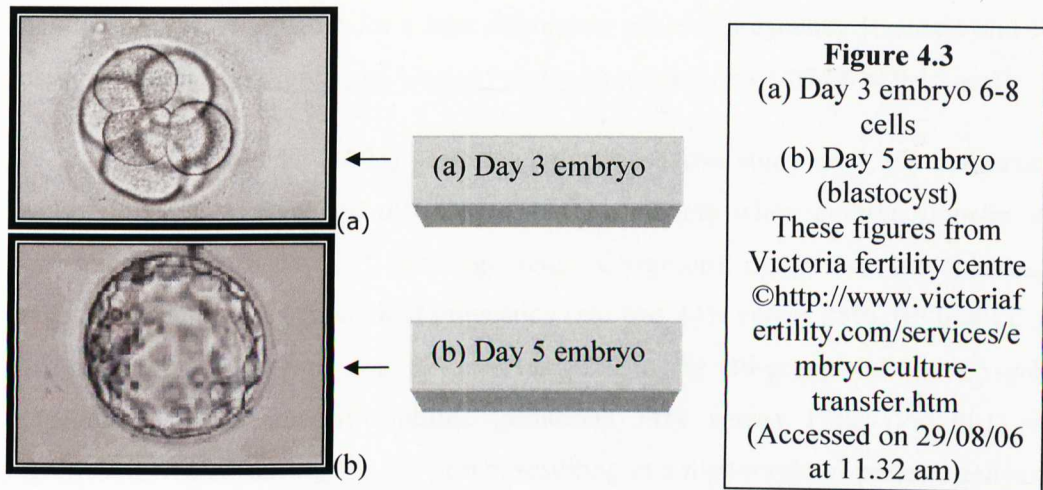
Blastocyst transfer has advantages and disadvantages. The advantages include the following: Firstly, allowing the embryos to develop in the laboratory for a longer period of time seems to be a better method for selecting the most normal embryos that would be more likely to implant. Secondly, the transfer occurs closer to the natural time when the uterine lining may provide a better environment for the embryo to implant. Because of the high pregnancy and implantation rates after blastocyst transfer, fewer embryos need to be transferred to maintain an acceptable pregnancy rate. This technique significantly reduces the chance of higher order multiple gestations such as triplets [© (Genetic and IVF institute http://www.givf.com/blastocyst.cfm (Accessed on 01/07/06 at 9:15 am)]

The risk and disadvantages of blastocyst transfer include the following: Firstly, 40% of grade 1 and 35% of grade 2 cleaving embryos have growth arrest before blastocyst formation (Edwards and Brody 1995). In this report, two fifth of the embryos formed blastocysts containing from 10 to >60 nuclei on day 5. At best only 13% of all fertilized eggs will form a normal blastocyst (Winston et al. 1991). Therefore, the total number of embryos available for transfer and freezing will be less. Unfortunately, some couples undergoing IVF, especially those with a low starting number of embryos, will have all their embryos die in the laboratory and will not have any embryos available for transfer. There is no evidence whether or not the embryos that die in the laboratory would have developed into a normal pregnancy if they had been transferred into the uterus at an earlier stage (Gardner et al. 2000). Secondly, a risk which is similar to transferring embryos at earlier stages, is that the blastocyst may not survive the freeze-

thaw process when utilized for a later attempt to achieve pregnancy [Genetic and IVF institute ©http://www.givf.com/blastocyst.cfm (Accessed on 01/07/06 at 9:15 am)]

Schwärzler et al. (2004) performed a retrospective study on 1,259 consecutive cycles, in order to evaluate differences in the outcome when embryo transfer was performed either on day 2–3 (cleavage stage, CS-group), or on day 4–5 (blastocyst stage, BS-group). They found that pregnancy rate was 44% versus 28%, (P<0.001), and the 'take home baby rate' was 37% versus 22% in the BS-group and the CS-group respectively.   The rate of multiple gestations 34% versus 17%, (P<0.001) was significantly higher among the BS-group, resulting in a higher rate of preterm deliveries < 36 weeks 26% versus 17%, P<0.045. Female factors causing infertility 40% versus 21% (P<0.001) was significantly higher among the BS-group. For the CS-group, the rate of singleton pregnancies 83% versus 66%, (P<0.001) and idiopathic cause of infertility 34% versus 22%, (P<0.012) were significantly higher. In addition, they found that there was no statistically significant difference in the sex, Caesarean section rate, Apgar score, umbilical artery pH-values, total mean birth weight, admission rate to intensive care unit, days of hospitalization or number of minor and major birth defects. They concluded that blastocyst transfer might lead to a higher pregnancy rate with an overall better take-home baby rate (Schwärzler et al. 2004), see table (4.2).

**Table 4.2** Difference in the outcome between embryo transfer at cleavage stage or at blastocyst stage. [This table is from Schwärzler et al. (2004)]

| Variable | Total | Cleavage-group | Blastocyst-group | P-value |
|---|---|---|---|---|
| Total number of transfers | 1259 | 549 | 710 | |
| Pregnancy rate | 468 (37%) | 156 (28%) | 312 (44%) | <0.001 |
| Miscarriages | 77 (16%) | 32 (21%) | 45 (14%) | n.s |
| Stillbirths | 6 (1%) | 3 (2%) | 3 (1%) | n.a. |
| Babies per implanted embryo (THBR) | 385 (31%) | 121 (22%) | 264 (37%) | <0.001 |
| Singleton in vital pregnancy | 274 (71%) | 100 (83%) | 174 (66%) | 0.001 |
| Twins in vital pregnancy | 107 (28%) | 20 (17%) | 87 (33%) | 0.001 |
| Triplets in vital pregnancy | 4 (1%) | 1 (1%) | 3 (1%) | n.s. |
| Total of multiple pregnancies | 111 (29%) | 21 (17%) | 90 (34%) | 0.001 |

Menezo et al. (1999) reported that statistically significantly more male infants were born after transfer of fresh blastocysts. No specific differences in birth weight were observed between infants born after blastocyst transfer compared with those born after spontaneous conception.

Blastocysts have been grown in vitro for many years, with results as good as 50% of embryos reaching the blastocyst stage in studies more than 10 years ago, although many failed to expand fully (Fishel and Edwards 1983). Expanded and hatching blastocysts were also cryopreserved, ready for a later transfer (Cohen et al. 1985; and Fehilly et al. 1985).

Clinical trials of blastocyst culture and transfer have largely focused on patients with a good prognosis, with an adequate response to gonadotrophin. However, Marek et al. (1999) performed a retrospective study for all patients who attended a fertility clinic and underwent blastocyst culture and transfer. They determined that extended culture resulted in increased implantation and pregnancy rates compared to the use of cleavage-stage embryo transfer. The implantation rates where 32.4% versus 23.3% respectively, P <0.05, pregnancy rates where 57.5% versus 46.1% respectively. In addition, there was a significant increase in the percentage of patients who did not have an embryo transfer 6.7% versus 2.9% respectively. Furthermore, the number of embryos transferred on day 5 was significantly below that transferred on day 3 (Marek et al. 1999). Similar results have been observed by Vidaeff et al. (2000), and they concluded that blastocyst transfer, with resultant high implantation rates, represents an effective means of eliminating high order multiple gestations in good prognosis patients and should be considered especially for patients electing to have a single embryo transferred (Marek et al. 1999; Vidaeff et al. 2000).

## *4.1.4 Influence of the time employed in the performance of the transfer:*

A number of studies have shown how some environmental factors can have a detrimental effect on embryos, such as exposure to light (Smith 1993; Evans et al. 1999) or extremes of temperature (Rocha et al. 1998). The importance of keeping embryo temperature close to 37°C and $O_2$ and $CO_2$ concentrations within specific ranges is well known (Bavister 1995; Cohen et al. 1997).

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Matorras et al. (2004) investigated whether there was any clinical significance to the time interval between embryo loading and embryo deposition. They found that the longer the duration the lower the pregnancy and implantation rates. They found that the decrease in pregnancy and implantation rates was gradual until the time interval was 120 seconds, after which it decreased sharply. They recommended speeding up the embryo transfer process wherever possible. A time interval of >120 seconds carries a poor prognosis and should, when possible, be avoided, see table (4.3)

**Table 4.3** The significance of the time interval between embryos loading and embryo depositing [This table is from Matorras et al. (2004)]

| Time elapsed between embryo loading and embryo deposition | <30 sec | 31-60 sec | 61-120 sec | >120 sec | P |
|---|---|---|---|---|---|
| Pregnancy rate (%) | 38.9 | 33.2 | 31.6 | 19.1 | <0.05 |
| Implantation rate (%) | 21.2 | 15.4 | 15.9 | 9.4 | <0.01 |
| Pregnancy rate excluding difficult transfers (%) | 40.0 | 33.3 | 32.0 | 19.4 | <0.05 |
| Implantation rate excluding difficult transfers (%) | 21.4 | 15.4 | 16.2 | 8.8 | <0.01 |

## 4.1.5 Site of placing the embryos in the uterus:

The clinical touch method was first described by Steptoe and Edwards (1978). With this method the catheter was gently inserted in to the uterine cavity until it touched the fundal endometrium, then withdrawn 0.5cm before the embryo was expelled.

Webster (1986) suggested placing the embryo about 10mm below the fundus. Nazari and co workers found that there was an increased incidence of ectopic pregnancy with high fundal transfers (Nazari et al. 1993), while Bennett and co workers found that there was an increased incidence of cervical pregnancy with low uterine transfers (Bennett et al. 1993). Depositing the embryos in the mid fundal area of the uterus was found to be important in improving the pregnancy rate (Rosenlund et al. 1996).

Lesny and co workers considered that not touching the endometrium and uterine fundus when transferring the embryo into the lumen of the endometrial cavity was most

important, and they demonstrated that touching the fundus with the transfer catheter stimulated junctional zone contractions (Lesny et al. 1998a). It had been shown already that these contractions have negative effect on the pregnancy rate (Fanchin et al. 1998b). To avoid this, it was suggested to deposit the embryos 1cm from the fundus and 2cm from the internal cervical os (Lesny et al. 1998a).

Naaktgeboren et al. (1998) suggested placing the embryos lower in the uterine cavity, and being absolutely sure that the catheter has passed through the cervical canal. Mansour and Aboulghar emphasised that a soft catheter can curve inside the cervical canal, and this can be confirmed by rotating the catheter 360°. They said that if it recoils this means that it is curved inside the canal. They also suggested taking individual measurements of the cervical canal and uterine cavity before attempting any embryo transfer (Mansour and Aboulghar 2002).

A study described by Coroleu et al. (2002) performed ultrasound guided embryo transfers on 180 patients who were randomised into three groups according to the site of placement of the embryos. In the first group, the embryos were placed 10±1.5mm from the fundus, while in the second and third groups the embryos were placed at 15±1.5mm, or 20±1.5mm respectively. They found that the implantation rates in groups 1, 2 and 3 were 20.6%, 31.3% and 33.3% respectively. They concluded that the depth of embryo placement into the uterine cavity may influence the implantation rate (Coroleu et al. 2002), see table (4.4). In contrast Franco et al. (2004) found that implantation and pregnancy rates were similar whether the embryos were deposited in the upper or in the lower half of the endometrial cavity.

**Table 4.4** Pregnancy rate in relation to the site of placing the embryo.
[This table is from Coroleu et al. (2002)]

| Pregnancy rate in day one | Pregnancy rate |
|---------------------------|----------------|
| 10 ± 1.5 mm | 20.6% |
| 15 ± 1.5 mm | 31.3% |
| 20 ± 1.5 mm | 33.3% |

### *4.1.6 Withdrawal of the embryo transfer catheter:*

Embryo transfer should be performed gently in order to avoid stimulation of junctional zone contractions (Lesny et al. 1998a). Martinez et al. (2001) performed a randomised study on 100 patients, which investigated the influence of the time interval before withdrawal of the embryo transfer catheter. They investigated whether this time interval could affect the pregnancy rate. They divided the patients into two groups: in one group, the catheter was gently withdrawn immediately, while in the second group the catheter was withdrawn after a 30 second wait. They found that there was no difference in the pregnancy rate in a population of women with a good response to stimulation and who had an easy ultrasound-guided transfer of good quality embryos. They concluded that, either this waiting interval was not sufficient to create a difference or the retention of the transfer catheter after embryo transfer was not a factor that affected the pregnancy rate (Martinez et al. 2001).

## *4.2   Is Bed rest necessary for the patient after embryo transfer?*

When Steptoe was performing embryo transfer more than 25 years ago, he used to keep the patient in the knee chest position on a special bed with a bumper. The patient would be instructed to slide forward on her abdomen and was advised to stay in bed that night and to try to avoid turning over. Subsequently embryo transfer became an out patient procedure with most women having only few minutes rest  (Slikke and Corson 2002)

http://www.obgyn.net/displaytranscript.asp?page=/avtranscripts/AIT2002_Corson (Accessed on 14.07.06 at 3.24pm)

Knutzen et al. (1992) performed a study on 34 infertile patients in the luteal phase of the cycle immediately preceding IVF treatment at the time of mock embryo transfer. They observed the uterine retention of radio opaque dye mimicking embryo transfer. The transfer was performed before they positioned the patient supine or axial for those who had a retroverted uterus or knee chest position for those who had an anteverted uterus. They then monitored the position of the dye during and after catheter removal and during patient roll over. They found that the dye remained in the uterine cavity in 68% of optimum embryo transfer positions, and in 48% of non optimum

embryo transfer positions. Of these two groups, the dye moved to the tubes, cervix and vagina in 38.2%, 11.8% and 8.8% respectively. They concluded that if the transfer had been an actual transfer, 32% of patients positioned optimally and 52% of patients positioned non optimally would have lost their opportunity to conceive due to the technique itself rather than the position of the patient (Knutzen et al. 1992).

A randomised controlled study performed by Botta and Grudzinskas (1997) found no statistical significant difference in pregnancy rates between patients who had 20 minutes bed rest following embryo transfer, compared to those who had 24 hrs bed rest. The same result has been obtained by other studies (Sharif et al. 1998; Rezabek et al. 2001; Amarin and Obeidat 2004) who found that the early mobilization groups after embryo transfer have a better implantation and pregnancy rate, a better take home baby rate and no statistical significance in the rate of twin pregnancy and miscarriages.

**Table 4.5(a)** Effect of bed rest after embryo transfer on the pregnancy rate. This table and the following tables 4.5(b,c,d and e) are from Natalija Vedmedovska, (2005), see ©www.gfmer.ch/PGC_RH_2005/pdf/Mobilization_ET_R.pdf (Accessed on 09/09/06, at 8.10pm)

| Study | 24 hrs bed rest after ET | 20-60 minute bed rest after ET | RR 95% CI | P |
|---|---|---|---|---|
| Botta and Grudzinskas 1997 | 21.6% | 21.3% | | >0.05 |
| Sharif et al. 1998 | 22.9% | 30% | | |
| Rezabek et al 2001 | 22.2% | 50% | | 0.08 |
| Amarin and Obeidat 2004 | 18% | 22% | 0.87-1.41(1.11) | >0.05 |

**Table 4.5(b)** Effect of bed rest after embryo transfer on the take home baby rate

| Study | 24 hrs bed rest | 20-60minute rest | P |
|---|---|---|---|
| Rezabek et al. 2001 | 11% | 40% | P < 0.07 |

**Table 4.5 (c)** Effect of bed rest after embryo transfer on the implantation rate / cycle

| Study | 24 hrs bed rest after ET | 20-60 minutes bed rest after ET | RR 95%CI | P |
|---|---|---|---|---|
| Botta and Grudzinskas 1997 | 24.1% | 23.6% | | >0.05 |
| Rezabek et al. 2001 | 14.5% | 22.5% | | 0.26 |
| Amarin and Obeidat 2004 | 9% | 14.4 | (1.10-1.47) 1.27 | <0.05 |

**Table 4.5(d)** Effect of bed rest after embryo transfer on twin pregnancy

| Study | 24 hrs rest after ET | 20-60 min rest after ET | RR 95% CI | P |
|---|---|---|---|---|
| Botta and Grudzinskas 1997 | 14.2% | 13.6% | 1.50 | >0.05 |
| Amarin and Obeidat 2004 | 9% | 20% | (1.03-2.19) 1.50 | <0.05 |

**Table 4.5(e)** Effect of bed rest after embryo transfer on the miscarriages rate

| Study | 24 hrs bed rest after ET | 20-60 min. rest after ET | RR 95% CI | P |
|---|---|---|---|---|
| Botta 1997 | 19% | 18.1% | | >0.05 |
| Amarin and Obeidat 2004 | 5% | 9% | 1.31(1.31-1.78) | <0.05 |

The authors of these studies advised early mobilization of the patient after embryo transfer, and they concluded that this was cost effective and might relieve some maternal stress. (Sharif et al. 1998; Rezabek et al. 2001; Amarin and Obeidat 2004).

## 4.3 Sexual intercourse around the time of embryo transfer:

Couples trying for a pregnancy tend to make love less frequently than those who have no concern about fertility. The reasons for this include the mistaken idea that a

long period of abstinence improve the sperm, and that the days around the time of ovulation are the only times when intercourse should take place. These ideas are exacerbated by anxiety or the fear that intercourse will dislodge an early implanting embryo (Lamont and Anderson 1993).

Intercourse might impair implantation by two mechanisms: the introduction of infection, or initiation of uterine contraction (Tremellen et al. 2000). Fox et al. (1970) found that uterine myometrial activity increase during intercourse. It was shown that these contractions might interfere with the implantation of an early embryo, since a high level of these contractions had a negative effect on the IVF-ET outcome (Fanchin et al. 1998b). In addition, it was found that sub-clinical infection of the upper genital tract is associated with a poor IVF-ET outcome (Fanchin et al. 1998a).

In animal studies, evidence shows that seminal plasma is important for achieving embryo implantation and development, and embryos transferred without exposing them to seminal fluid have a lower rate of implantation (Queen et al. 1981). In the human, studies are small and inadequately randomised. Some show seminal plasma to be beneficial (Bellinge et al. 1986; Marconi et al. 1989), whereas others have shown no effect (Fishel et al. 1989; Qasim et al. 1996).

Tremellen et al. (2000) performed a study on 1,343 embryos transferred during 478 cycles of IVF and found that there was no significant difference between intercourse and abstinence in relation to pregnancy rate 23.6% and 21.2% respectively, but the proportion of embryos that were viable at 6-8 weeks was significantly higher in women exposed to semen compared to those who abstained (11.01 versus 7.69 viable embryos per 100 transferred embryos, $P<0.036$, OR 1.48, and 95 % CI 1.01-2.19 ). They concluded that exposure to semen around the time of embryo transfer increased the chance of successful early embryo implantation and development (Tremellen et al. 2000).

## 4.4 Ways of making embryo transfer easier:

### 4.4.1 Mock embryo transfer before actual embryo transfer:

Over the past 10–15 years, there have been increasing success rates with assisted reproductive technologies (ART) in all age groups. The Society for Assisted

Reproductive Technology reported an increase in live birth rates from 28% in 1996 to 32% in 2002 (National Summary and Fertility Clinic Reports 2001 ; Neithardt et al. 2005). This increase has been attributed to multiple factors including improved ovarian stimulation protocols (Bergh et al. 1997), advances in laboratory techniques (Scoutt 2002), and improvement in ET techniques (Choe et al. 2001).

There are many studies, which have suggested carrying out a mock embryo transfer before actual ET (Mansour et al 1990; Knutzen et al 1992; Sharif et al. 1995; Ghazzawi et al. 1999). Mock embryo transfer enables the selection of the most suitable catheter, thus limiting the time the embryos spend out of the incubator.

Mansour and co workers suggested a mock embryo transfer in the cycle before the start of IVF treatment (Mansour et al. 1990), while Sharif and co workers suggested a mock embryo transfer on the day of egg retrieval, or just before the actual embryo transfer, so as to avoid the problems of a different position of the uterus on the day of the actual embryo transfer (Sharif et al. 1995).

Mansour and co workers compared the outcome between two groups; the first group was subjected to mock embryo transfer, while the second group was not. They found that there was no difficulty at the time of embryo transfer in the first group, while in the second group; embryo transfer was difficult in 29.8%. They found also that the pregnancy and the implantation rates in the first group were 22.8%, and 7.2% respectively, while in the second group, the pregnancy and the implantation rates were 13.1% and 4.3% respectively. They concluded that mock embryo transfer is a simple, painless procedure, which will give an idea about the length of the uterine cavity, the degree of the utero cervical angle and the presence of any cervical stenosis, and determine the most suitable catheter and technique for transfer. Moreover, they explained the significantly lower pregnancy rate in the second group as due to difficult embryo transfer. Furthermore, they suggested mock embryo transfer for every patient in order to avoid unexpected difficulty and failed embryo transfer (Mansour et al. 1990). Similar results were obtained by Sharif et al. (1995). They performed mock embryo transfer with a full bladder just before the actual embryo transfer, and found that the pregnancy rate was 45.1% for the mock ET group and 20.6% for the non mock ET group.

Henne and Milki (2004) in their study found that 98% of the anteverted uteruses (AV), and 45% of the retroverted uteruses (RV), will remain in the same position at the time of actual embryo transfer in fresh cycles, while 88% of the AV, and 67% of the RV, will remain in the same position at the time of actual embryo transfer in subsequent frozen cycles, see table (4.6).

**Table 4.6** Difference between uterine positions at the time of mock ET and actual ET. [This table is from Henne and Milki (2004)]

| Total cycles at fresh Mock ET | AV at actual ET | RV at actual ET | Total cycles at thawed Mock ET | AV at actual ET | RV at actual ET |
|---|---|---|---|---|---|
| 623 AV | 608 (98%) | 15 (2%) | 114 AV | 100(88%) | 14(12%) |
| 213 RV | 118 (55%) | 95(45%) | 46 RV | 15(33%) | 31(67%) |

Mansour and Sharif demonstrated that the transfer technique is basically simple, but unexpected difficulty can be encountered during the transfer procedure at the level of the internal os, and they explained that this can be caused by extreme flexion of the uterus or by stenosis of the cervical canal (Mansour et al.1990; Sharif et al. 1995).

Knutzen and co workers advocate mock embryo transfer in a cycle prior to the actual IVF cycle, in order to find the most suitable catheter for each patient, avoid unpredicted difficulty and reduce the number of difficult transfers (Knutzen et al. 1992).

## 4.4.2 Ultrasound guided embryo transfer:

Each step of the IVF process has been analysed intensively in an attempt to improve the success rate, but embryo transfer has not been given the same amount of attention that other aspect of the process have. Recently it has become apparent that this area is probably one of the most important areas to be considered. It is clear that embryos should be transferred to the uterus in an atraumatic way, and that they should be placed in an area most likely to afford implantation.

Most IVF centres still rely on clinical feel as a tactile method for transfer of the embryo within the uterine cavity. The possible use of ultrasound to facilitate embryo transfer was first reported by Strickler and co workers. They reported that ultrasound–guided transfers were easier, with less catheter distortion, and ejection of the transfer

bubble could be documented. This was reassuring to both clinician and patients (Strickler et al. 1985; Leong et al.1986).

A prospective randomised study performed by Wisanto et al. (1989) compared different embryo transfer catheters. One of the groups had ultrasound guided embryo transfer using a TDT catheter and they found this group had a significantly improved pregnancy rate (Wisanto et al. 1989). Others have advocated the use of ultrasound guidance during the transfer procedure to improve the pregnancy rate. Hurly et al. (1991) found that the pregnancy rate was improved by the use of transvaginal guidance in a single embryo transfer. Prapas et al. (1995) also reported a significant improvement in the pregnancy rate in ultrasound guided embryo transfer.

Woolcott and co worker emphasised that blind embryo transfers may be unreliable, and the outer guiding catheter abutted, or either indented or embedded in the endometrium in 17.4%, 24.8%, and 33.1% respectively. Furthermore, in 7.4%, there was tubal transfer, and they also confirmed in their study the maintenance of air bubble position after the patient stood up after embryo transfer. In addition, they concluded that ultrasound guidance had many potential advantages. It facilitated the placement of the soft catheter, avoiding touching the fundus and, in cases with long cervix, it would confirm that the catheter was beyond the internal os (Woolcott and stanger 1998).

Lewin et al. (1997) showed that ultrasound would guide the embryo transfer catheter along the uterine cavity without damaging the endometrium, and it will avoid plugging of the catheter and avoid bleeding. Prapas et al. (2001) found that ultrasound assistance in embryo transfer on day 3 and day 4 significantly improved the pregnancy rate in IVF but had no impact on day 5 embryo transfers.

A randomised trial performed by Coroleu et al. (2000) found that the use of abdominal ultrasound guided embryo transfer improved the pregnancy rate from 33.7% to 50% . Sallam et al. (2002) measured the utero-cervical angle immediately before embryo transfer and then moulded the transfer catheter according to the degree of angulation. They found that this significantly increased the pregnancy and implantation rates and reduced the number of difficult and bloody transfers.

In all the previous studies, ultrasound was mainly used to confirm that the tip of the transfer catheter was correctly positioned near the uterine fundus. Two prospective trials done by AL-Shawaf et al. (1993) and Khan et al. (1999) failed to show significant improvement in the pregnancy rate with the use of ultrasound. Similar results were achieved by Anderson et al. (2002) who carried out ultrasound-guided embryo transfer and compared the result retrospectively with IVF cycles when ultrasound was not used. They found that there was no difference in the outcome, and the improvement in the pregnancy rate was in patients who had previous failed IVF cycles. See figures 4.4 and 4.5 (a and b).
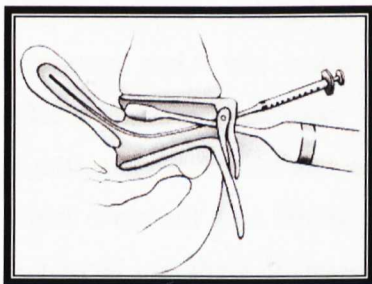


**Figure 4.4**
Ultrasound guided embryo transfer. This figure is showing the site of placing the vaginal probe.
This figure is from Anderson et al. (2002)



(a)
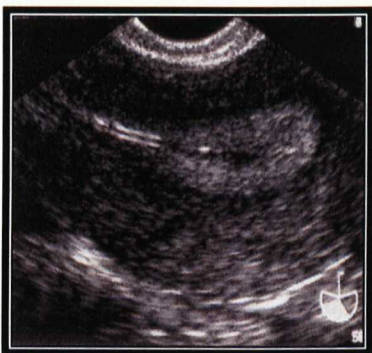
**Figure 4.5 (a)** Ultrasound guided embryo transfer. This figure shows the appearance of the ET catheter before transfer of embryos as viewed by transvaginal ultrasonography with the uterus in the sagittal plane. This figure is from Anderson et al. (2002)



(b)
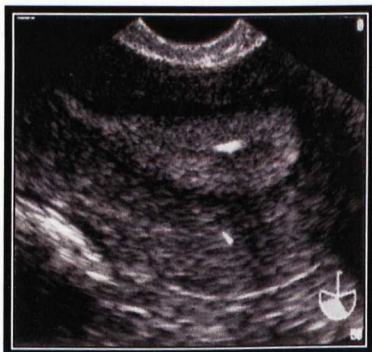
**Figure 4.5(b)** Ultrasound guided embryo transfer. This figure shows the air bubbles within the uterine cavity after transfer of the embryos. This figure is from Anderson et al. (2002)

### 4.4.3 The use of tenaculum in the embryo transfer procedure:

A tenaculum is used for correction of the uterine position in any gynaecological procedure, but in embryo transfer, the situation is different. When Steptoe and Edwards were performing embryo transfer 28 years ago, they did not use tenaculum so as to avoid pain to the patient (Edwards et al. 1980).

Lesny et al. (1999a) performed a study to establish whether it is safe or not to use a tenaculum in embryo transfer procedures. The study was performed at the time of mock embryo transfer, at mid cycle on the day when down regulation was started. The junctional zone contractions before and after the application of the tenaculum were measured, and it was found that there was a significant increase in these contractions after tenaculum application. The cervico-fundal, random, and opposing contractions all significantly increased and 4/20 of the patients developed fundo-cervical contractions, which were not seen before the application of the tenaculum. Fanchin et al. (1998b) suggested that these contractions might be responsible for the mechanical expulsion of the embryo from the uterus, and therefore have a negative impact on IVF–ET. Based upon their results, Lesny et al. (1999a) advised avoiding the use of a tenaculum during embryo transfer procedures.

Evidence shows that oxytocin can cause contractions in the non pregnant uterus, especially during orgasm. These contractions may be involved in the passive transport of sperm and the embryo through the oviduct (Russell and Leng 1998).

Dorn et al. (1999) measured the level of serum oxytocin before and after tenaculum application in 10 patient having embryo transfer. They divided these 10 patients in to 2 groups, one group with tenaculum application, and the other one with out. They performed serial measurements of oxytocin levels at 20-second intervals during embryo transfer. They found that oxytocin levels were significantly higher in 4 out of 5 patients in whom a tenaculum had been applied, while in the other group none of the patients had an increase in the level of oxytocin. They suggested avoiding a tenaculum application whenever possible in embryo transfer procedure.

### 4.4.4. Cervical dilatation for patients who have had a previous difficult embryo transfer:

The difficulty of the embryo transfer has been reported to affect the pregnancy rate in some centres (Leeton et al. 1982; Mansour et al. 1990).

Serhal et al. (2003) performed cervical dilatation with a hygroscopic cervical rod for patients who had either failed to conceive after a previous difficult ET or who had a difficult mock embryo transfer. The dilator was inserted for 4hrs prior to starting gonadotrophin stimulation. Subsequently 79% had an easy embryo transfer, and 55% become pregnant. It was concluded that cervical dilatation using a hygroscopic dilator facilitated difficult embryo transfer and helped to improve the pregnancy rate.

Prapas et al. (2004) examined the influence of cervical dilatation in patients who had had two previous failed IVF cycles. The dilatation was done 1-3 months before embryo transfer. They found that patients who had a cervical dilatation yielded significantly higher pregnancy rates than the non dilatation group 40% versus 24% respectively; (P<0.01). The same result was found for implantation rates 24.1% versus 14.9%; (P<0.01), and live birth rates 34.48% versus 19.56%; (P<0.01). It was concluded that cervical dilatation 1-3 months before embryo transfer led to an improved pregnancy rate (Prapas et al. 2004).

Different results were obtained by Groutz et al. (1997). They studied 41 treatment cycles in 22 patients who had had a previous difficult or impossible embryo transfer due to cervical stenosis. These patients had a cervical dilatation 48 hrs before embryo transfer. It was found that this cervical dilatation made embryo transfer easier in 39 cases, but the pregnancy rate was very low, only one clinical pregnancy (2.5%) and one ectopic pregnancy. We understood from the previous result that it is likely that cervical dilatation should be done several weeks before embryo transfer.

### 4.5 Factors which affect the technique of embryo transfer

The success of IVF treatment depends on maximizing the efficiency of each step of the procedure. Embryo transfer is the final step in the IVF treatment cycle. The goal of the transcervical embryo transfer is to deliver the embryos into the uterine fundus in

an atraumatic way, at a place where implantation is maximum, to avoid the presence of blood or mucus on the catheter tip, and avoid expelled or retained embryos. The most important thing to be avoided is the generation of junctional zone contractions (Craft et al. 1981).

This step is associated with success or failure, and failure of implantation is the most common cause of failed embryo transfer. Different interventions have been evaluated, including position of the patient (Jones et al. 1983; Reinthaller et al. 1986), quality of culture media (Martinez and Trounson 1986) and the type of catheter used (Hazout and Me'ne'zo 1996). There are many factors, which can affect the outcome of embryo transfer, these factors include the following:

## 4.5.1 Effect of difficult embryo transfer on the outcome of IVF:

Studies have been done trying to modify the embryo transfer procedure in order to achieve optimum results. Many studies have compared the outcome between difficult and easy procedures (Leeton et al. 1982; Wood et al. 1985; Egan et al. 1990; Tur-Kaspa et al. 1998; Mansour et al. 1990; Lesny et al. 1999). Some of these studies showed that difficult embryo transfers have no effect on the outcome, (Wood et al. 1985; Egan et al. 1990; Tur- Kaspa et al. 1998), while other studies showed that difficult embryo transfers negatively influenced the pregnancy outcome (Leeton et al. 1982; Mansour et al. 1990).

Evidence shows that there is a significant increase in the junctional zone contractions after tenaculum application in embryo transfer procedures (Lesny et al. 1999a), and they suggest the avoidance of the use of tenaculum so as to avoid the increase in the serum level of oxytocin (Dorn et al. 1999). This means avoiding unnecessary manipulation whenever possible because these contractions have negative effects on the implantation of the embryo (Fanchin et al. 1998b).

In a retrospective study done by Tomas and co-workers, the study included 4,807 patients who had had a recent embryo transfer. Patients were grouped into those who had had easy, intermediate, and difficult embryo transfers. They considered the procedure as difficult if it was time consuming, or if there was a need to change the catheter, or to do a cervical dilatation, or if there was blood on the catheter tip. They

found that there was a significant difference in the outcome between the easy, intermediate, and difficult procedures. The pregnancy rate was 30.3% for the easy and the intermediate and 21.1% for the difficult ones. They concluded that difficulty of embryo transfer is an independent variable for predicting the outcome of IVF-ICSI embryo transfer procedure (Tomas et al. 2002).

Marconi et al. (2003) performed direct visualisation of endometrial lesions immediately after mock embryo transfer using different kinds of embryo transfer catheters. They assessed the endometrial lesions by the use of micro hysteroscopy. In their study, they compared the endometrial lesion after the use of Tomcat, Wallace, and Frydman catheters in 23 infertile patients, who had their mock transfer on day 2-5 post ovulation. They found that there were different endometrial lesions ranging from tunnel like, groove like, punch like, to crater like lesions, and they found that the Wallace catheter was less traumatic to the endometrium than the Tomcat catheter. The Frydman catheter caused the most significant lesions, and they suggested that some of these lesions might be capable of compromising the success of embryo transfer; they concluded that the severity of some of the lesions observed might explain the low pregnancy rate associated with the difficult or traumatic embryo transfer procedures (Marconi et al. 2003).

There is a case reported by Napolitano and Fonttis (2002) in which a 32 year old infertile patient had 5 oocytes retrieved, IVF resulted in only one zygote and cleaving embryo and a difficult, traumatic and bloody transfer was attempted on day 3. The transfer procedure was cancelled that day as the embryo was retained in the catheter, so it was kept in the incubator in culture media until a blastocyst was formed. A second transfer was performed and a successful pregnancy was achieved and a healthy baby was born. It was suggested that in case of difficult embryo transfer, waiting for the blastocyst stage might allow a second non traumatic transfer with a better outcome when the embryo and the uterus are not under stress (Napolitano and Fonttis 2002).

## 4.5.2 Effect of the type of embryo transfer catheter on the outcome of the procedure:

The ideal embryo transfer catheter should avoid any trauma to the cervix, or to the endometrium (Ghazzawi et al. 1999). Embryo transfer catheters may be classified in general into three different types: preloaded, after loaded and catheter with memory (Edwards and Brody 1995). The choice of the catheter remains technique-dependant, and it is an important decision to make.

About 80% of patients undergoing IVF reach the embryo transfer stage (HFEA 1996) but only a small proportion of them achieve pregnancy, and the pregnancy rate after embryo transfer is dependent upon multiple factors including embryo quality, endometrial receptivity and the technique of embryo transfer itself (Mansour and Aboulghar 2002).

Various groups have described their favourite ET techniques (Craft et al. 1981; Leeton et al. 1982; Edwards et al 1984) and many different embryo transfer catheters are commercially available, mainly composed of non-toxic plastics and/or metal. They vary in length, calibre, location of the distal port and degree of stiffness and malleability. These catheters can be subdivided by the material they are made of (i.e. metal, hard or soft plastics) and whether they are equipped with or without an introducing cannula that facilitates the transfer procedure (Abou-Setta et al. 2005).

Initiation of soft catheter use began with the Frydman catheter (Laboratories CCD Paris France), and evolved to almost exclusive use of the Wallace catheter (Copper Surgical, Shelton, CT, USA). Both catheters possess stiffer outer sheaths that stabilize the softer inner cannula, which carries the embryos to the endometrial cavity for transfer (Wood et al. 2000).

In the early 1980s, Edwards and co workers were the first to use the Wallace catheter (Edwards et al. 1984) and they described it as a soft flexible catheter surrounded by a movable outer sheath to give support if needed.

The Tefcat catheter (Cook Ob-Gyn, Spencer, IN, USA) or Tom Cat (Sherwood Medical, St Louis, MO, USA) have a relatively stiff, hard, polyethylene or Teflon sheath that enters the endometrial cavity to deposit the embryos.

Several surveys have shown that the transfer catheter ranks high as an important variable (Wisanto et al. 1989; Grunert et al. 1998; Amorcho et al. 1999; Ghazzawi et al. 1999) and some rate the type of catheter used as high as the third most important variable (Kovacs1991).

Studies by many authors have compared soft with firm embryo transfer catheters (Wisanto et al. 1989; Grunert et al. 1998; Amorcho et al. 1999; Ghazzawi et al. 1999). These studies showed that softer embryo transfer catheters overall performed better. Al Shawaf et al. (1993) found no difference in the performance of Wallace and Frydman with regard to the outcome. Other authors found that Tom Cat catheters yielded a significantly higher pregnancy rate than the Frydman catheter 28% versus 16%; P = 0.03 (Gonen et al. 1991).

Hans et al. (2002) performed a randomised controlled study comparing two groups of patients, in one group they used TDT rigid catheter and in the other group, they used K soft cannula (Cook K soft 5000 trans universal embryo transfer set), the K soft cannula consist of single lumen cannula with 12.5 cm a rigid proximal part and 4cm soft distal part. The transfer catheter was made of an undisclosed soft polyurethren material. They found that the type of the embryo transfer catheter contribute significantly to the success of the IVF program.

Marconi and co workers found that there were different kinds of endometrial lesions caused by the use of different kinds of transfer catheters, and they explained the difference in the outcome with the use of different catheters as due to the degree of trauma caused by these catheters. They suggested that some of these lesions might be capable of compromising the success of embryo transfer (Marconi et al. 2003).

To gain better understanding of each patient anatomy, a mock embryo transfer is performed in most IVF centres. One advantages of performing a mock ET is that the direction and the length of the uterine cavity can be measured (Sharif et al. 1995). This

facilitates an atraumatic transfer by choosing the most suitable catheter for each patient (Mansour et al. 1990).

Microbial contamination of the embryo transfer catheter tip is correlated with a significant reduction in pregnancy rate (Egbase et al. 1996; Fanchin et al. 1998a). Prophylactic antibiotics administered at the time of oocyte retrieval significantly reduce the incidence of positive microbial cultures from embryo transfer catheter tips 48 hours after antibiotic administration (Egbase et al. 1999).

## 4.5.3 The presence of blood on the tip of the transfer catheter:

There is evidence which shows that the presence of blood on the catheter tip is associated with a low pregnancy rate after embryo transfer (Goudas et al. 1998; Awonuga et al. 1998). Other evidence shows that the embryo can be retained by either blood or mucus occluding the catheter (Leeton et al. 1982; Egan et al. 1990).

Awonuga et al. (1998) found that gently cleaning away the cervical mucus from the end of the ET catheter would prevent retained embryos, especially embryos that have undergone assisted hatching, while (Edwards et al. 1984; Visser et al. 1993; and Ebner et al. 2001) did not find statistically significant effect of catheter contamination by blood or mucus on the outcome, see table (4.7).

**Table 4.7** Effect of the presence of blood on the tip of embryo transfer catheter. [This table is from Ebner et al. (2001)]

| The parameters which may affect the outcome of ET | Pregnant | Non pregnant | Implantation rate | Pregnancy rate |
|---|---|---|---|---|
| Blood on the catheter | | | | |
| Yes | 18 | 38 | 16.2 % | 32.1 % |
| No | 87 | 161 | 16.9 % | 35.1 % |
| Mucus on the catheter | | | | |
| Yes | 11 | 22 | 19.3 % | 33.3% |
| No | 94 | 179 | 16.4 % | 34.7% |

## 4.5.4 Is it necessary to remove the cervical mucus?

Mansour et al. (1994) carried out mock embryo transfers by using methylene blue in place of transfer media in a similar volume. They found that when cervical

mucus was aspirated before mock embryo transfer, methylene blue was present at the cervix in 23%, as compared to 57% of those with out aspiration. They concluded that cervical mucus affected the rate of embryo expulsion into the cervix.

Nabi and co workers (1997) carried out a non randomised study for 1,204 patients and found that embryos were significantly more likely to be retained in the transfer catheter when it was contaminated with mucus, as compared with embryo transfer procedures when the catheters were not contaminated, 17.8% and 3.3% respectively; (P<0.000001), and they suggested that cervical mucus can plug the tip of the embryo transfer catheter, causing difficulty in delivering the embryos inside the uterine cavity and thus increasing the risk of a retained embryo. Furthermore, they demonstrated that the embryos can stick to the cervical mucus around the catheter, especially with a small amount of culture media, and can be dragged outside during withdrawal of the catheter (Nabi et al. 1997).

Other workers found that flushing of the cervical canal with embryo culture media using a small syringe of 1ml to be associated with an improvement of the clinical pregnancy rate. McNamee et al. (1999) performed a retrospective study and found that patients who had a vigorous lavage had a 55% pregnancy rate, and 26% implantation rate, compared with a 41.7% pregnancy rate and a 10.4% implantation rate in a control group, see table (4.8).

Sallam et al. (2000) performed an RCT and found that there was no statistically significant difference with or without flushing of the cervix with pregnancy rates 25.5% and 34.5%; (P=0.4053), and implantation rates 15.38% and 17.46 %;( P=0.7687).

**Table 4.8** Effect of the vigorous cervical lavage on the outcome of IVF-ET.
[This table is from McNamee et al. (1999)]

| Effect of vigorous lavage of the cervix | Pregnancy rate | Implantation rate |
|---|---|---|
| McNamee et al 1999 | | |
| Yes | 55% | 26% |
| No | 41.7% | 10.4% |
| Sallam et al 2000 | | |
| Yes | 25.5% | 15.38% |
| No | 34.5% (P=0.4053) | 17.46% (P=0.7687) |

## *4.5.5 Effect of the junctional zone contractions on the outcome of embryo transfer:*

Junctional zone contractions are present at frequencies in the region of 4 contractions per minute (Fanchin et al. 1998b). Fanchin and co workers found that the pregnancy and implantation rates decreased as the frequency of these contractions increased. Based on this result, the researchers were trying to find a way to decrease these contractions in order to improve the implantation and the pregnancy rate, so they started to analyse the embryo transfer procedure intensively.

Many studies have investigated junctional zone contractions during embryo transfer (Nabi et al. 1997; Ghazzawi et al. 1999; Wisanto et al. 1999; Lesny et al. 1999; Sallam et al. 2000; Tomas et al. 2002). They all suggest that embryo transfer should be done in a gentle way, avoiding any excessive manipulation of the cervix, and not touching the fundus (Lesny et al. 1999).

## *4.5.6 Effect of different operators on the outcome of embryo transfer:*

The embryo transfer technique has remained the same since it was described by Steptoe et al in 1978 although many studies were done trying to improve the outcome of IVF-ET (Wisanto et al. 1999; Lesny et al. 1999; Aboulghar et al. 1999; Fanchin et al. 1998a; 1998b; Mansour and Aboulghar 2002). ET was analysed step by step and one of the variables which was evaluated was the experience of the operators.

Visser et al. (1993) found that there is no difference in the pregnancy rate between different operators. The same result was obtained by Barber et al. (1996) and Karande et al. (1999).

Hearns et al. (2000) performed a study on 854 infertile patients. All of them received the same regime of down regulation, ovarian stimulation and luteal support. Embryo transfer was performed by 11 different physicians in rotation, so that there was no patient selection. There was a significant difference in the pregnancy rate between physicians but on direct observation of the physician with the lowest pregnancy rate, there was no obvious difference in their technique.

## 4.5.7 Nurses performing embryo transfer:

In the past, embryo transfer was done exclusively by doctors. More recently, nurses have started to perform the procedure after proper training and doctors will perform the procedure only if the nurses find difficulty at the time of mock embryo transfer, or if they are on vacation. Barber et al. (1996) describes a study by the Oxford Group of 771 embryo transfers. 88% were performed by nurses, and 12% by doctors, either as first operators, or after the nurse has experienced difficulty. The nurse-transfer clinical pregnancy rate was 36.2% and the doctor-transfer rate was 29.4%, P >0.05 (Barber et al. 1996).

Sinclair et al. (1998) compared the outcome of 151 ETs performed by doctors with 371 procedures performed by a nurse. The pregnancy rate per nurse transfer was 40.2% versus 41%, with corresponding implantation rates of 16.9% versus 17%. None of the differences were statistically significant (>0.05), they showed that nurses managed to do 71% of the embryo transfers.

## 4.5.8 Effect of fluid accumulation within the uterine cavity at the time of embryo transfer on the outcome of IVF-ET:

Fluid accumulation within the uterine cavity, or hydrometra, after ovarian stimulation, and before embryo transfer, has only sporadically been reported in the literature (Welker et al. 1989; Mansour et al. 1991; Andersen et al. 1994; Sharara and McClamrock 1997), and most of these cases were also claimed to have hydrosalpinx. How often this complication occurs in women with tubal infertility is unknown, neither is whether women with other indications for IVF have the same problem.

Studies have been done to investigate the correlation of hydrometra with tubal disease and its effect on the outcome of IVF-ET. Many authors explained that embryonal apposition could not occur when a fluid layer is overlaying the endometrium (Andersen et al. 1994; Sharara. and McClamrock 1997).

Endometrial cavity fluid is occasionally observed during assisted reproductive technology (ART) cycles; however, few reports have described its prevalence or significance. The use of ultrasound to evaluate the endometrial cavity for fluid accumulation prior to embryo transfer has been reported by many studies (Mansour et

al., 1991; Andersen et al. 1996; Bloechle et al. 1997) but this is still not performed systematically in most centres.

Andersen et al. (1996) evaluated 38 patients with a hydrosalpinx who underwent one IVF cycle, 17 (44.7%) of whom had a history of hydrometra. Only one woman (5.8%) conceived. Three patients (8%) had fluid in the endometrium prior to hCG, and none of these conceived after embryo transfer. Six additional patients (16%) were noted to have fluid in the endometrium 5 days after embryo transfer and only one conceived, but with an ectopic pregnancy. They also found that there was no difference in the largest diameter of the hydrosalpinx between those patients who conceived (7/38) as compared to those who did not (31/38) and there were also no differences in the endometrial pattern between the two groups.

Sharara and McClamrock (1997) reported on two cases with hydrosalpinx. In both cases the hydrosalpinx enlarged in response to gonadotrophin stimulation, hydrosalpinx fluid reflux into the endometrial cavity became radiologically visible only after receiving hCG. From the first case, a clear yellowish fluid was aspirated transvaginally from the hydrosalpinx until it collapsed completely. In addition, 100 µl of clear fluid was aspirated from the endometrial cavity until it had disappeared by ultrasound. After extensive counselling, the patient elected to proceed with the embryo transfer if there was no fluid re-accumulation prior to transfer. Six assisted hatched embryos were replaced because of her age, and to increase the implantation rate, but no pregnancy ensued. Then she underwent laparoscopic salpingectomy prior to initiating another cycle, during which five embryos were replaced, which resulted in an ongoing singleton gestation, see figure 4.7 (a).

In the second case, a Wallace catheter was introduced through the cervical os and 100 µl was aspirated with disappearance of the fluid collection observed by ultrasound. The patient was extensively counselled against undergoing an embryo transfer, and opted to cryopreserve the four resulting embryos at the pronuclear stage (Sharara and McClamrock 1997), see figure 4.7 (a), and (b).

Levi et al. (2001) observed the accumulation of endometrial fluid in some patients during stimulations and in the others after hCG administration, and the overall incidence was 8.2%. They found that when endometrial fluid was observed during

stimulation, the cancellation rate due to poor ovarian response was significantly higher 29.8% versus 16.9%, (P<0.05), and the pregnancy rate per started cycle was significantly lower 26.3 versus 42.4%, (P <0.05), than cycles without endometrial fluid. While for those who had endometrial fluid only after hCG administration, the pregnancy rates were similar to the group for which endometrial fluid was not observed (Levi et al. 2001).

**Table 4.9(a)** Effect of intrauterine fluid accumulation at the time of ET.
[This table is from Levi et al. (2001)]

|  | Endometrial fluid observed at the time of stimulation | Endometrial fluid not observed at the time of stimulation | P |
|---|---|---|---|
| Cancellation rate | 29.8% | 16.9% | <0.05 |
| Pregnancy rate / started cycle | 26.3% | 42.4% | <0.05 |

Chien et al. (2002) carried out a retrospective study of 746 cycles, and found that uterine fluid accumulation was identified in 35 cycles (4.7%). 18/225 (8%) had tubal disease, and 17/521 (3.3%) had no tubal disease. In 15 out of 35, cycles (2%) the fluid persisted until the time of embryo transfer, 2 out of 20 cycles of women with transient fluid accumulation were pregnant and non of those with fluid retention on the day of embryo transfer conceived. The authors concluded that fluid accumulation in the uterine cavity detected during the IVF cycles had a detrimental effect on the embryo implantation (Chien et al. 2002), see table 4.11(b).

**Table 4.9(b)** Effect of intrauterine fluid accumulation on the outcome of IVF.
[This table is from. Chien et al. (2002)]

| Total cycles | Cycles with fluid accumulation in the uterus | Cycles with tubal cause | Cycles with no tubal cause | Cycles with temporary fluid accumulation | Cycles with persistent fluid accumulation |
|---|---|---|---|---|---|
| 746 | 35 ( 4.7% ) | 18/225 8% | 17/521 3.3% | 20 cycles 2 (10%) were positive | 15 cycles all were negative |

Akman et al. (2005) retrospectively investigated patients who had intrauterine fluid accumulation, and had either PCOS or tubal factor infertility. This group was compared with another group of patients with either PCOS or tubal factor who had ovarian stimulation but did not show any fluid collection. It was found that implantation rates were lower in the tubal factor patients in the presence of endometrial fluid 6.12% and 21.4%, respectively in comparison with all other tubal factor infertile patients in whom no fluid accumulation inside the cavity was detected. Things were different in the PCOS group as there was no significant difference in the implantation rate in patients with endometrial fluid in comparison with the other cycles in which no fluid accumulation was detected. It was concluded that when fluid collection inside the endometrial cavity is first seen during ovarian stimulation of PCOS patients undergoing IVF, embryo transfer can be performed safely if the fluid has disappeared and not returned by the day of embryo transfer, while in tubal factor cycles one should think of either cancellation of the cycle or cryopreservation of all embryos (Akman et al. 2005).

If fluid accumulation is found before hCG administration, cancellation of the cycle should be considered. Mansour et al. (1991) recommended consideration of evacuation of the fluid noted after hCG administration, but other studies have reported re-collection of the fluid immediately after aspiration (Bloechle et al. 1997).

Kato, Sharif and co workers recommend transmyometrial embryo transfer as an alternative method when fluid accumulation is noted before embryo transfer, (Kato et al. 1993; Sharif et al. 1996), yet the effectiveness is unproven. Cryopreservation of embryos until a favourable cycle is the treatment of choice at the present time, but it has to be proved in a further study.

**(a)**
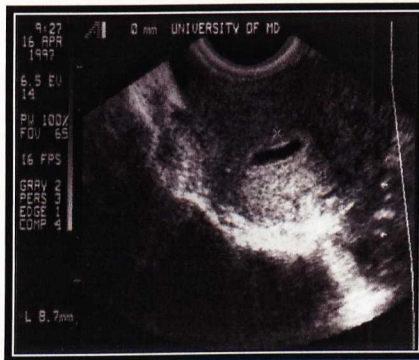


(b)

**Figure 4.6** Endometrial fluid collections noted after human chorionic gonadotrophin (hCG)

**(a)** Case 1; the endometrial lining measured 13 mm. The fluid collection measured 4.0 mm.

**(b)** Case 2, in which endometrial fluid collection noted after hCG measured 4.5 mm.

This figure is from Sharara and McClamrock, (1997).

In conclusion, fluid accumulation within the uterine cavity during IVF treatment mainly occurs in patients with tubal infertility. However, it can also be observed in patients with non-tubal factors. Although it is not a common complication of IVF–ET cycles, the presence of excessive uterine fluid is detrimental to embryo implantation. Serial transvaginal ultrasonography evaluation of both the endometrium and uterine cavity is necessary during the entire treatment cycle to avoid transferring embryos into an unfavourable uterus.

## 4.6  Is their any limit to the number of IVF-ETcycles which can be attempted on the same patient?

The difficult question, which always needs to be asked, is what to do if IVF fails, and how many times the couple can try again? Before answering this question, first of all, the IVF cycle should be evaluated carefully, and the record of the embryologist and ovarian reserve should be assessed again. In patients with a low

ovarian reserve, their chance to become pregnant is low (Aboulghar et al. 1999). A diagnostic laparoscopy might find endometriosis, and surgical treatment can improve the outcome (Marcoux et al. 1997).

There is a case report of 37 attempts over 12 years to achieve pregnancy (Kovacs and Hewlett 2004). The patient was diagnosed with unexplained sub fertility at the age of 29 years. Her assisted reproductive treatment started in April 1991, through which she had eight stimulated IVF cycles, twenty-five clomifene minimum stimulated IVF cycles, three natural IVF cycles and one frozen thawed embryo transfer cycle. She had a total of twenty six embryos, transferred in twenty two attempts. Sixteen days after her third egg retrieval she developed a pelvic abscess and ovarian hyprestimulation syndrome followed by adult respiratory distress syndrome. She was treated conservatively and the subsequent egg retrieval was performed under antibiotic cover. In 1992, she had a laparoscopic resection of a hydrosalpinx. In 1998, she had a hysteroscopic resection of a uterine fibroid, and during her last attempt, in March 2002, she became pregnant, but her pregnancy was complicated by intermittent heavy vaginal bleeding starting from eight weeks up to twenty weeks. She had caesarean section at 34 weeks for meconium stained liquor. They suggested trying again and again until treatment becomes successful.

There is evidence, which shows that genetic abnormality is more common among couples with repeated IVF failures (Raziel et al. 2002). Others attributed implantation failure to immunological abnormalities, and they suggested that couples with repeated IVF failures and unexplained infertility should undergo genetic counselling and testing, and if genetic problem are identified donor gamete transfer should be considered (Balasch et al. 1996).

Kovacs (2002) suggested that if a genetic abnormality is only present in some of the cell lines (mosaicism), repeated IVF could be recommended after proper counselling. If genetic problems are identified, either donor gametes should be considered or preimplantation genetic diagnosis can be used to evaluate the embryo. Farhi et al. (2000) suggested that ZIFT can be considered as a mode of treatment for patients with repeated failure of implantation in IVF-ET.

## 4.7 Protocol for embryo transfer based on factors

1. Mock embryo transfer at the time of down regulation, so that if there is any sign of cervical stenosis, dilatation can be performed to make ET easier.

2. There is no convincing evidence that patient position affects the outcome of embryo transfer (Diedrich et al. 1989). The dorsal lithotomy position is most commonly used for embryo transfer, especially for patients with a retroverted uterus, whereas for patients with an anteverted uterus, the knee–chest position has been recommended to eliminate expulsion of embryos from the uterus (Knutzen et al. 1992).

3. Gentle insertion: manipulate the cervix with a speculum; avoid any excessive manipulation to avoid initiation of uterine contractions.

4. Remove excess cervical mucus, and flush the cervix (but not the canal) with culture media.

5. Transabdominal ultrasonographic guidance with a full bladder or transvaginal ultrasonographic guidance to avoid the catheter tip causing any damage to the endometrium, and avoid touching the fundus.

6. A full bladder can make ET easier by making the cervico-uterine angle straight.

7. Soft catheter, 20-30μL volume of culture media with two air bubbles to avoid spillage of the embryo. Inject the embryo slowly and remove the catheter slowly

8. Catheter should be checked by the embryologist for any retained embryo

# CHAPTER FIVE

# *The Uterine Junctional Zone and its Contractions*

## *5.1 Histology and embryological origin of the myometrium:*

The human uterine muscular wall is composed of three layers; the stratum subvasculare, immediately adjacent to the endometrium with a predominantly circular arrangement of the muscular fibres, the subserosal stratum supravasculare, with a predominantly longitudinal arrangement of the muscle fibres, and in between, the stratum vasculare, which consists of a three dimensional mesh of short muscular bundles, and constitutes the bulk of uterine muscular wall in the female (Wetzstein 1965).

During human embryology both the stratum supravasculare and the stratum vasculare develop in the third trimester of gestation or even postnatally, while the inner myometrium is already apparent at the beginning of the second trimester (Werth and Grusdew 1898).

With the circular arrangement of mesenchymal cells around the primordial uterus and tubes, ontogenetically early formation of the inner circular layer is also documented by a kind of fundo cornual raphea of the stratum subvasculare, which results from the fusion of two paramesonephric ducts, and their mesenchymal element, which together form the primordial uterus (Werth and Grusdew 1898).

The origin of the outer myometrium is from the outer mescenchyme "non mullerian origin", whiles the origin of the endometrium, and the stem cells of the junctional zone, are both paramesonephric "mullerian origin" (Werth and Grusdew 1898).

The sub endometrial layer of the myometrium "the junctional zone" is characterized by high cellular density, lower cytoplasmic/nuclear ratio and different expressions of the components of the extra cellular matrix (Brosens et al. 1998).

The differentiation of the myometrium into two distinct layers is strictly under ovarian hormonal control, see figure (5.1). In the pre-menarcheal girl, and postmenopausal woman, the zonal anatomy is often indistinct. Ovarian down regulation with a GnRH analogue "Gonadotrophin Releasing Hormone analogue" leads to the appearance of the uterus mimicking that of the postmenopausal period on MRI scan (Demas et al. 1985; and Wiczyk et al. 1988).



**Figure 5.1** MRI appearance of the human uterus.
From inside to outside on the level of the uterine cavity: the endometrium with high signal intensity; the stratum subvasculare of the myometrium with low signal intensity (`junctional zone'), the stratum vasculare of the myometrium with high but more irregular signal intensity and the stratum supravasculare of the myometrium with intermediate signal intensity. The halo comprises about one-quarter of the thickness of the whole myometrium on the level of the uterine corpus.
[This figure is from Kunz et al. (2000)]

The endometrium and the junctional zone are also functionally different from that of the outer myometrium. The function of the outer myometrium is mainly to expel the foetus during labour, while the endometrium and the junctional zone are involved in many of the other biological activities of the reproductive system, including

particularly sperm transport, and implantation. Proliferation and differentiation of the endometrium prepares for implantation of the embryo, and peristalsis of the junctional zone may assist the passive transport of sperm, and the embryo in the oviduct leading to a higher implantation of the embryo in the uterus. Peristaltic movements may also help to remove the endometrial debris during menstruation (Kunz et al. 1996); and (Leyendecker et al. 1996). For all of these functions to be possible the epithelium and the stroma of the endometrium, as well as the junctional zone, constantly undergo structural and biochemical changes through out the ovarian cycle (Guidice and Ferenczy 1996).

## 5.2 Immunocytochemical expression of oestrogen, progesterone, and oxytocin receptors in the human myometrial and endometrial layers:

Immunocytochemistry is a method of detecting the presence of specific proteins in cells and tissues. With special monoclonal antibodies, it allows visualisation of the oestrogen and progesterone receptors in an individual cell and the tissue layers of the uterus as a target organ of ovarian steroids (Einspanier et al. 1998).

Studies have been performed on the uteruses of animals to see the distribution of the oestrogen, progesterone, and oxytocin receptors in different phases of the ovarian cycle. This research found that the staining of the steroid receptors was mainly intranuclear, whereas the oxytocin receptor was localised in the cytoplasm or on the outer cell membrane (Einspanier et al. 1998).

Einspanier et al. (1998) also found that staining, localization, and intensity varied, as did the percentage of immunoreactive cells, throughout the cycles. Similar studies were done for the human uterus, but in most of these studies only the sub endometrial layer of the myometrium was analysed and was considered to be representative of the whole uterine muscle wall (Lessey et al. 1988; and Snijder et al. 1992).

Noe et al. (1999) performed Immunocytochemistry on the uteruses of 27 women and found that there was no consistent pattern of steroid receptor expression across the myometrial wall. The expression of steroid receptors in the sub endometrial

layer of the myometrium paralleled the cyclical pattern of the endometrial epithelium and stroma, while the outer myometrium showed no cyclical pattern at all but a strong staining throughout the whole cycle, see figure (5.2).
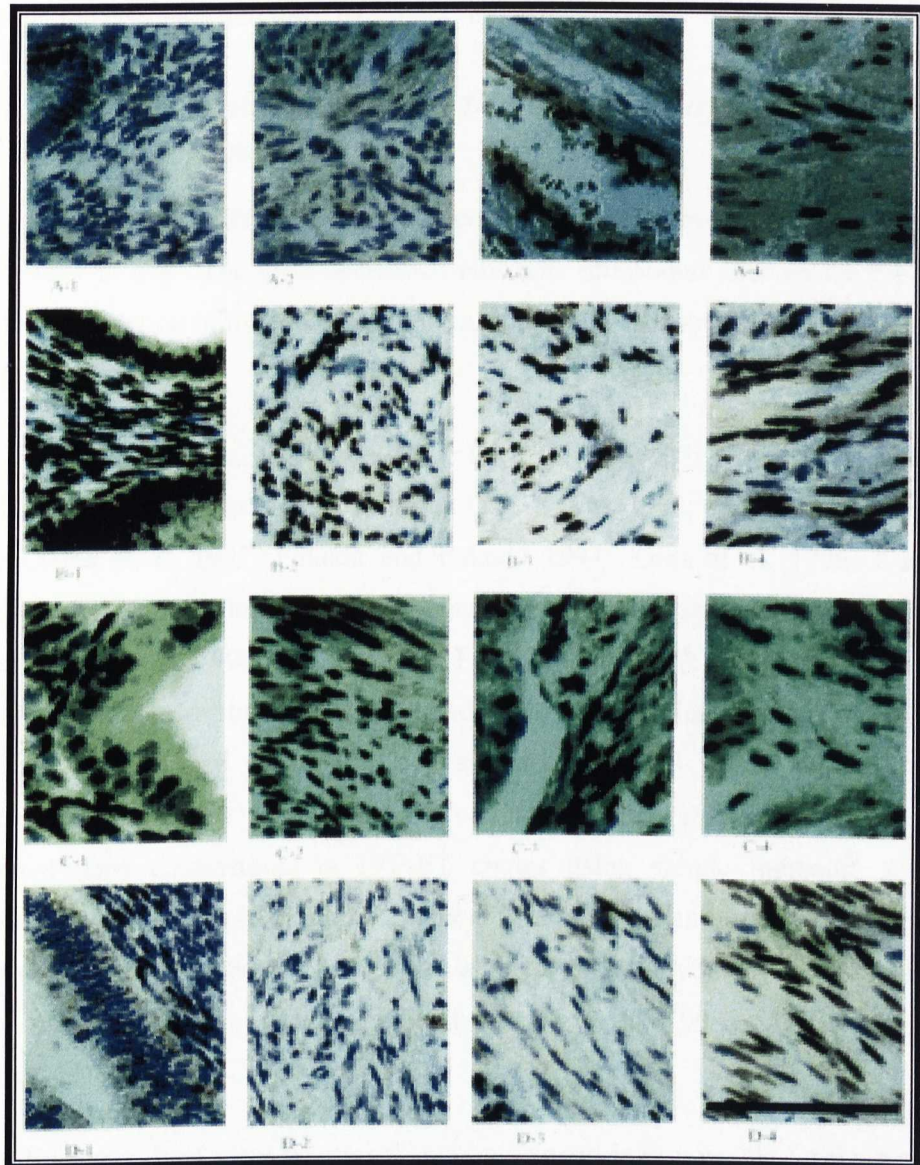


**Figure 5.2..** Representative sections of Immunostaining for oestrogen receptors of the uterine wall throughout the menstrual cycle. The sections depict the endometrium (**1**), the stratum subvasculare (**2**), the stratum vasculare (**3**) and the stratum supravasculare (**4**) respectively, during the early proliferative (**A**), peri-ovulatory (**B**), early secretory (**C**) and late secretory (**D**) phases respectively of the menstrual cycle. Bar = 50 μm. [This figure is from Noe et al. (1999)]

# 5.3 The use of imaging techniques in the detection of junctional zone:

Due to their non-invasive nature imaging techniques are ideally suited to assessing the endometrium prior to embryo transfer.

## 5.3.1. The use of ultrasound in the detection of `junctional zone and its contractions

In contrast to MRI, ultrasound is much cheaper and more widely available while being easier to use. The development of real time ultrasound allowed the rapid non-invasive observation of the junctional zone as a hypo dense zone about 3-5 mm in depth (Kunz et al. 1998b).

Junctional zone movement has been identified by both transabdominal (Brinholz 1984) and transvaginal ultrasound (Oike et al. 1988; Abramowitz and Archer 1990; Lyons et al. 1991; Fukuda and Fukuda 1994; Kunz et al. 1996; Kunz and Leyendecker 1996). Junctional zone contractions have been quantified in several studies (Ijiland et al. 1996; 1997a; 1997b; Fanchin et al. 1997; and Lesny et al. 1998a) and it has been shown that both natural and fertilization cycles contain different kinds of contractions (Ijiland et al. (1997; Lesny et al. 1998a). Early studies done by Abramowitz and Archer (1991); and Fukuda and Fukuda (1994) were able to show junctional zone contractions in IVF-ET cycles using simple methods. With the introduction of more sophisticated equipment "audio visual and computer technology" it became possible to observe this particular layer of the myometrium in more detail and to see its movement more objectively (Lesny et al. 1998a; and Fanchin et al. 1998b).

Evidence shows that junctional zone movement is responsible for the peristaltic movement of the endometrium (Scoutt et al. 1991; Turnbull et al. 1994; Brosens et al. 1995; and Tetlow et al. 1997).

Ultrasound has been described as an easy way to detect the contractions of the junctional zone but its use might affect the outcome. Lesny and co-workers have listed several factors that might affect the result. Firstly, pressing the vaginal transducer down too hard with the hope of getting a better image of the endometrium might disturb

the contractions. Secondly, the type of contractions seen depends on the view itself, whether it is longitudinal, transverse or oblique, with the optimum method of seeing the contraction being through the use of the longitudinal view. In a longitudinal view the direction will be observed correctly "cervico-fundal or fundo-cervical" while in the transverse view the contractions will involve the whole circumference of the endometrium and appear like a contracted ring. In the oblique view, the image will be a mixture of both the longitudinal and the transverse views. Moreover, with the use of the fast forward function of the video tape machine fine contractions cannot be visualised (Lesny et al. 1998a). The above finding may be responsible for the inconsistency in the classification of uterine contractions (Abramowitz and Archer 1990; Lyons et al. 1991).

## 5.3.2. The use of magnetic resonance in the detection of uterine junctional zone:

Several studies have shown that the structural differences of the myometrium allow in vivo visualization of the myometrial zonal anatomy by T2 weighted MRI (Lee et al. 1985; Brown et al. 1991; Scoutt et al. 1991). In women of reproductive age distinct zonal differentiation in both the corpus and the cervix may be identified. Using these method three layers can be distinguished in the myometrium. Surrounding the high signal intensity of endometrial strip there is a low signal intensity junctional zone followed by an outer intermediate signal intensity of the intermediate zone and a thin low signal intensity subserosal zone, see figure (5.1), and (5.3). The junctional zone looks like a hypo dense halo surrounding the hyper-dense area of the endometrium (Lee et al. 1985; Brown et al. 1991; and Scoutt et al. 1991).

The appearance of the junctional zone is subjected to a wide variety of physiological factors including direct mechanical effect due to contraction or hormonal effect (McCarthy et al. 1986; Togashi et al. 1993). Ovarian suppression with GnRH analogues lead to an appearance of the uterus mimicking that of post menopause on MRI, while HRT in post menopausal women results in reappearance of the myometrial zonal anatomy (Demas et al. 1985).

Turnbull et al. (1995) showed that myometrial zonal anatomy changes profoundly during pregnancy. Focal disruption of the junctional zone occurs in early pregnancy, observed as early as 7 days post ovulation. Other authors found that during pregnancy the junctional zone increases in signal intensity and the zonal difference become indistinct with normal zonal anatomy reappearing within 6 months of delivery (Willms et al. 1995).

Kido et al. (2005) evaluated the effect of oral contraceptive pills on the junctional zone in 23 women who were of reproductive age and using oral contraceptive pills (OCP users). The outcome was compared to that of 15 women who were not using oral contraceptive pills (controls). Results showed that the endometrium and junctional zone were significantly thinner and that the myometrium was thicker in the OCP users compared to controls. Uterine peristalsis was markedly decreased in the OCP users and the signal intensity of the myometrium and the cervical mucus was significantly higher in the oral contraceptive users than in controls (Kido et al. 2005).

The uterus exhibits subtle and rhythmical contractions with the inner myometrium showing subtle wave like movements while the direction and the incidence of the peristalsis varies according to the menstrual cycle (Lyon et al. 1991). Such uterine peristalsis may be displayed using the cine mode display of ultra fast MRI (Nakai et al. 2000). Since disorders of these peristaltic movements may possibly affect the chance of implantation (Fanchin et al. 1998), or may result in a variety of clinical disorders such as endometriosis, ultra fast MRI might become the appropriate procedure to identify relevant uterine function in the future (Nakai et al. 2000).



(a)    (b)
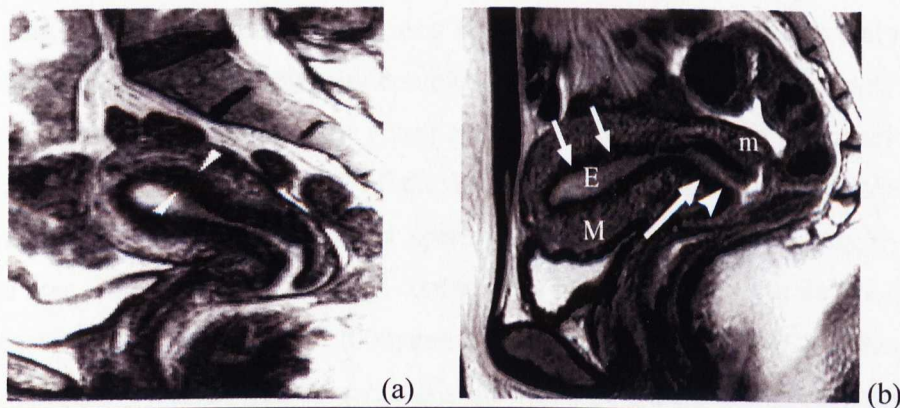
**Figure 5.3**
MRI detection of the junctional zone (a), and (b). MRI of human uterus, the junctional zone involves both the fundus and the cervix. From Nakai et al. (2001)

### 5.3.3. The use of cine mode MRI for the detection of junctional zone contraction:

Conventional MRI techniques may take more than a minute to obtain a T2 weighted image of the uterus and as such the time resolution of this image may be out of the range of that of the peristalsis. Therefore, junctional zone contractions have been previously disregarded by MRI (Fujiwara et al. 2003).

MRI techniques have rapidly advanced and cine mode MRI is one of the recent advances that show some promise. Nakai et al. (2001) demonstrated that this new tool could be used to allow them to obtain serial images of the uterus with an examination time of less than a second. As such more than 100 images can be obtained within only a few minutes. Cine mode display of these serial images in the same plane can provide demonstrable information relating to the dynamic changes of the human abdomen and pelvic organs. This information was impossible to obtain through the use of traditional static MRI techniques or the use of CT scanning of the pelvis (Nakai et al. 2001; and Fujiwara et al. 2003).

Nakai and co-workers found that the use of ultra fast imaging techniques allowed clear identification of the uterine peristalsis as a wave conduction of a low intensity area within the inner myometrium or as an endometrial stripping movement Nakai et al. (2001). In addition, they found that there are no identifiable diurnal changes in these contractions and that the direction and the frequency are dependent on the phase of the menstrual cycle. Furthermore, they found that during menstruation the low intensity movement of the endometrium is conducted form the fundus to the cervix. The peak frequency of contractions was found to be during the peri-ovulatory phase with sub endometrial low intensity conduction from the cervix to the fundus, while in the luteal phase these contractions disappear. The direction and the frequency of these contractions fitted the functions of the uterus. They concluded that cervico-fundal contractions might be responsible for sperm transport and retaining the embryo in the fundal area, while the fundo-cervical contractions may be responsible for the discharge of menstrual blood (Nakai et al. 2000; and 2001). Other authors noted that the direction and the frequency of the uterine peristalsis shown in Cine mode MRI are in accordance with those seen in transvaginal ultrasound, which are 2-3 contractions per minute (de Vries et al. 1990; and Oike et al. 1990).

## *5.4 Contraction of the non-pregnant uterus:*

The uterus is not a quiescent organ and contractions of the non-pregnant uterus throughout the ovarian cycle have been described Martinez et al. (1973). This group studied these contractions by the use of both invasive and non-invasive methods. These included monitoring the amplitude and the frequency of contractions by measuring the intra-uterine pressure. They used three rubber balloon catheters to record the intra-uterine pressure and they distinguished between propagated and non-propagated contractions. The results of the study noted that the frequency of these contractions is highest in the peri-ovulatory phase of the ovarian cycle Martinez et al. (1973).

There is evidence to show that in the non-pregnant uterus oestrogen receptor expression is highest in the late follicular phase and decreases sharply in the early luteal phase while progesterone receptor expression has an initial rise in the follicular phase followed by a constant level throughout the cycle (Snijder et al. 1992). Other evidence has shown that the expression of oestrogen receptors is significantly higher in the sub endometrial myometrium. However, the underlying mechanism is unknown (Richard and Tiltman 1995).

It has been shown that the junctional zone exhibits a cyclical pattern of oestrogen and progesterone receptor expression that is parallel to that of the endometrium, while the outer myometrium does not exhibit a cyclical pattern of oestrogen or progesterone receptor expression (Noe et al. 1999), see figure (5.2).

It has been shown that during the reproductive years the lymphoid aggregate consists mainly of T lymphocytes and few B lymphocytes. This lymphoid aggregate is found mainly at the endometrio-myometrial junction (Stewart et al. 1992). Recently, it was found that CD3-positive T lymphocytes, which are found in characteristic lymphoid aggregate in the endometrio-myometrial junction, play an important role in polarizing the endometrial epithelium by producing cytokines such as gamma interferon (Tabibzadeh et al. 1988). The mechanism underlying the cyclical pattern of inner myometrial contractions is not well understood though it is thought that the synchronous contractions of the inner myometrium depend on the electrical coupling of the smooth muscle by gap junctions, which are intercellular communication channels consisting of protein, in the human myometrium. The protein involved in gap

junctions is mainly connexin-43 and the expression of these gap junctions is mediated through the binding of certain proteins (Geimonen et al. 1996). In the myometrium, oestrogen receptor activation leads to increased concentration and stabilization of these proteins (Webb et al. 1995). The higher level of oestrogen receptor expression in the junctional zone during the follicular phase may be explained by oestrogen regulation of connexin-43. Progesterone and human chorionic gonadotrophin are thought to inhibit the expression of these gab junctions and this may explain the cyclical pattern of these contractions (Rao and Ambarus 1994; and Zhao et al. 1996).

The presence of these gap junctions alone is not enough to trigger myometrial contraction. It was found that gap junctions, with the help of several cytokines such as epidermal growth factor, might modulate myometrial contractions (Gardner and Stancel 1989). Binding sites for other uterotonic substances, such as endothelin-1, have also been found recently and it has been suggested that endothelin-1 in the endometrium can induce release prostaglandin F2 alpha (Bacon et al. 1995). It is postulated that junctional zone differentiation is regulated by complex interactions between ovarian sex steroid hormones and cytokines along with uterotonin produced locally at the endometrio-myometrial junction (Brosens et al. 1998).

## 5.5 The junctional zone as a separate functional unit:

The junctional zone is the myometrial layer just underlying the endometrium and has recently been described as a separate functional unit. One of the functional properties of this layer is that it produces contraction waves during the natural cycle (Ijiland et al. 1996; 1997b; Kunz and Leyendecker 1996). These contractions have been classified according to their direction as: contractions directed toward the uterine fundus (cervico-fundal), contractions from the fundus to the cervix (fundo-cervical), random contractions, and opposing contractions (Ijiland et al. 1996).

It has been described that the lower endometrium remains mobile throughout the entire cycle and that different contractile patterns are associated with higher pregnancy rates (Ijiland et al. 1997a). It has also been demonstrated that these contractions may be strong enough to displace embryos in the uterine cavity (Lesny et al. 1998a).

It was found that the direction and the amplitude of these contraction waves are stimulated by hormonal influence (Kunz et al. 1996), and physical stimuli (Lesny et al 1998b; Lesny et al. 1999a). High frequency contractions on the day of the embryo transfer during IVF/ET treatment were found to reduce the chances of implantation and pregnancy (Fanchin et al. 1997).

## 5.6 Junctional zone contractions in the natural cycle:

Spontaneous endometrial wave-like activity of the uterus during the natural cycle was observed during the 1970s (Martinez et al. 1973), but the velocity and the duration of the wave interval throughout the cycle were not initially reported. Advances in transvaginal tomography enabled researchers to see the endometrial wave-like movements in more detail. These advances therefore generated an interest in the possible clinical significance of the endometrial wave-like movement (Lyons et al. 1991; Fukuda and Fukuda 1994; Ijiland et al. 1996; 1997a; 1997b).

It has been shown that uterine contractions of the non-pregnant uterus involve only the sub endometrial layer of the myometrium and that these contractions exhibit cyclical patterns of frequency and direction Lyons et al. (1991). Further, recently it was found that peristaltic activity of the non-pregnant uterus plays an important role in the process of reproduction and that an impairment of these contractions may result in infertility Kunz et al. (1996); and (1997).

Observation of the endometrial wave-like movement has been performed by transvaginal ultrasound in 23 cycles of 16 healthy females with regular cycles (Ijiland et al. 1996). Serial ultrasound scanning was commenced on day 3 of the menstrual cycle and continued throughout all the phases of the cycle at 1-3 days intervals. The scanning was for 2 minutes and found that in the early follicular phase the endometrial movement could not be observed. From the late follicular phase, the endometrial like movement was gradually increased, and the direction was vertical to the longitudinal uterine axis. During the peri-ovulatory phase the intensity and frequency of endometrial wave-like movement reached a peak and almost all of these wave-like movements were directed from the cervix to the fundus. After ovulation, the appearance of the endometrial like movement gradually decreased and the direction of

the contractions becomes parallel to the longitudinal uterine axis. In the mid-luteal phase the endometrial like movement disappeared gradually only to reappear before menstruation with movement from the fundus to the cervix. There was also a trend of increasing wave velocity from the mid-follicular to the late-follicular phases. Cervico-fundal contractions have the highest velocity in the peri-ovulatory phase where the frequency is also highest and in the early luteal phase the wave interval may become negative (Ijiland et al. 1996; 1997a; and 1997b).

There is evidence to show that this endometrial wave-like movement is associated with myometrial contractions (de Vries et al. 1990) while other evidence shows that these myometrial contractions can be assessed by measurement of uterine cavity pressure (Akerlund et al. 1978; Gubbini et al.1991).

Measurement of uterine cavity pressure throughout the menstrual cycle has shown that the uterine cavity pressure is approximately 30mmHg in the peri-ovulatory phase and that it gradually decreases toward the mid-luteal phase when uterine cavity pressure is approximately 0mmHg (Csapo 1980). Uterine cavity pressure may reach up to 100mm Hg during menstruation and changes in the endometrial movement is synchronised with the changes in the uterine cavity pressure. From this, it was concluded that it is difficult to explain the underlying hormonal influence on this cyclical pattern of contractions (Csapo 1980). Evidence has shown that prostaglandin levels are high during menstruation while the level of oestrogen and progesterone is low (Downie et al. 1974).

Researchers have observed these endometrial like movements during the natural cycle and measured the levels of oestrogen and progesterone during their observation (Toshimichi et al. 2002). They found that these endometrial like movements are synchronised with the changes in the serum oestrogen level. They also found that these endometrial waves like movements disappear at the onset of increase of the progesterone level. The mechanism put forward to explain this is that prostaglandins contribute to the fundo-cervical contractions at the peri-menstrual phase while in the follicular phase prostaglandin levels will be reduced and then increase again in the mid-luteal phase. This means that the decrease in the contractions in this phase is not contributed to by the increasing level of prostaglandins, but is instead due to the

antagonistic effect of progesterone on prostaglandins (Toshimichi et al. 2002). Based on this evidence, they explained that the antagonistic effect of progesterone on prostaglandins contributed to the disappearance of the endometrial wave-like movement in the mid-luteal phase. They also found that there is a parallel relationship between endometrial wave-like movement and serum oestradiol level. This finding suggested that oestrogen might contribute to the endometrial wave-like movement in the peri-ovulatory phase (Toshimichi et al. 2002). Differing research study evidence appears to show that some endometrial wave-like activity may cause the hydrostatic alteration and turbulence in the uterine cavity fluid that is necessary for nutrient and oxygen supply to the peri-implantation embryo (Ijiland et al. 1996; Kunz et al. 1996). From the above findings, it is clear that junctional zone contractions reflect an important physiological phenomenon while cervico-fundal contractions contribute to the ascent of sperm in the female genital tract and to higher implantation of the embryo in the uterus (Kunz et al. 1996).

## 5.7 Uterine function in the transportation of sperm:

Transport of the spermatozoa within the female genital tract is one of the critical steps in the process of reproduction. To be successful this step requires a patent and functioning uterus and oviduct, as after ejaculation the sperm have to migrate from the site of deposition in the posterior vaginal fornix to the ampullary portion of the fallopian tube where fertilization will occur. Then the fertilised oocyte will have to be transported the opposite way to reach the uterine cavity for implantation. However, the mechanism for coordinating and timing this bidirectional transport is not understood (Harper 1994; and Pulkkinen 1995).

The mechanical patency of the oviduct may be assessed by procedures such as hysterosalpingogrphy (HSG), laparoscopy with dye chromo perturbation, and Hysterosalpingo Contrast Sonography (HyCoSy). However, none of these procedures will assess the functional integrity of the oviduct (Randolph et al. 1986; Kiyokawa et al. 2000).

Studies have been done to try to find a procedure that could asses the functional integrity of the oviduct (Kunz et al. 1996; Wildt et al. 1998). A 1998 study of a group

of women looked into the distribution of radioactive particles following insertion into the vagina Wildt et al. (1998). The women had tubal patency confirmed by the use of a Hysterosalpingogram and then the dominant follicle was identified and localised by ultrasound. Serum levels of oestrogen and progesterone were measured and 1-2 ml of micro labelled (technetium 99) human serum albumin was used (their size roughly corresponded to the size of spermatozoa). These small particles were injected in the posterior fornix and their distribution assessed with the use of a gamma camera. The researchers found that labelled particles could be detected as early as 2 minutes after intravaginal application, indicating that there had been rapid transport of the particles from the vagina into the uterus. The researchers also observed the uptake of the particles into the uterus during the follicular as well as during the luteal phase of the cycle, and they found that in the follicular phase 15% of the patients had the radioactive particles enter the fallopian tube bilaterally, in 64% the distribution was unilateral, and in 21% the radioactive particles remained in the uterine cavity with 6% of subjects showing a significant amount of the radioactive particles present in the peritoneal cavity Wildt et al. (1998). In addition, they found that the pattern of distribution of the radioactive particles within the uterus in the luteal phase is different from that observed during the follicular phase. In the luteal phase, there was a broad area while in the follicular phase there was an elongated area and transport to the oviduct Wildt et al. (1998). Furthermore, they found that with the increase in the diameter of the leading follicle the epsilateral sperm transport (to the site of the dominant follicle) was significantly increased from 10% to 75%. The researchers also found that this epsilateral sperm transport increased after oxytocin administration and that there is an increase in the tone and amplitude of the contractions as well as a reversal of the pressure gradient from a fundo-cervical to a cervico-fundal direction Wildt et al. (1998). Interestingly, the subsequent pregnancy rate following timed intercourse or intrauterine insemination was significantly higher among those who had epsilateral sperm transfer Wildt et al. (1998). The researchers concluded that the uterus and the fallopian tube act as a functional unit through action as a peristaltic pump. The activity of this pump will increase in the follicular phase and therefore increase the transport to the epsilateral side. They also suggested that oxytocin may have a critical role in this process Wildt et al. (1998). Another study has also suggested that there is an endocrine signal from the dominant follicle to the epsilateral site of the cornua Kunz et al. (1996).

The intensity and the direction of junctional zone contractions have been found to differ according to the stage of the cycle and that fundo-cervical contraction will disappear in the peri-ovulatory phase of the cycle and while there is an increase in the cervico-fundal contraction at the same phase has been shown by several studies (Ijiland et al. 1996; 1997a; 1997b).

Two theories of uterine sperm transportation have been put forward (William et al. 1993; Kunz et al. 1996). The first suggests that direct sperm transport is a genuine function of the uterus (William et al. 1993). This study excluded the mechanism of chemotaxis from the sperm transportation mechanism. The authors explained that direct sperm transport requires specific morphological structures to enable the uterus to transport the sperm into the tube. The other suggestion was that the uterus, although it is an unpaired organ, will behave as a paired organ according to the morphological division of the circular muscle of the stratum subvasculare "junctional zone" at the site of the fundus and the cornua and therefore increase the blood flow in the cornua at the epsilateral side in relation to the dominant follicle (William et al. 1993; Kunz et al. 1996). The same studies concluded that the utero-ovarian counter current system may provide higher oestradiol concentrations in the cornual region of the epsilateral side and that oestradiol may induce endocrine and paracrine changes (William et al. 1993; Kunz et al. 1996). It has also been found that oestradiol will cause upgrade regulation of the oxytocin receptors in the endometrium (Zingg et al. 1995). It has been suggested that this rapid transport of sperm within the genital tract could be mediated by these contractions (Kunz et al. 1996; 1998).

## 5.8 Uterine junctional zone contractions during assisted reproductive cycles

There is some evidence to show that the sub-endometrial layer of the myometrium, which consists of condensed myocytes, is responsible for the wave-like movement of the endometrium (Scoutt et al. 1991; Turnbull et al. 1994).

Junctional zone contractions have been observed in the natural cycle (Ijiland et al. 1996). Further work has also been carried out by Lesny and co workers in which they studied 18 female egg donors (Lesny et al. 1998a). Serial vaginal ultrasound scanning was performed for each woman for 3-5 minutes after down regulation. Scans

was done on day 0, day 8, day 10, the day of the hCG injection, before and after egg retrieval as well as days 2, 3 and 4 after egg retrieval. This research found that the junctional zone contractions were never seen at the time of down regulation and that they appeared in all cases on day 8 after super ovulation. The researchers observed many kinds of contractions, including fundo-cervical and random waves, but the most accentuated contraction was in the cervico-fundal direction. The same contractions were predominant at the time of hCG injection. Opposing contractions appeared while fundo-cervical and random contractions decreased. It was found that the cervico-fundal contractions increased in frequency, velocity and intensity immediately after egg retrieval. In addition, they saw that these contractions have wave-like pattern movement and may be disrupted by rapid random movement which was not seen prior to this stage. Furthermore, they found that on days 2 and 3 after egg retrieval these contractions were decreased and that on day 4 the endometrium was very quiet (Lesny et al. 1998a).

## 5.9 Effect of junctional zone contractions on the success of IVF-ET

Ijiland and co workers (1997a) did a prospective study for 59 infertile patients and they analysed the endometrial activity throughout the natural cycle, they found that conception cycles showed less-activity as compared with non conception cycles. Further study done by the same authors, regarding unidirectional waves (the fundo-cervical and the cervico-fundal waves) looked into the relation between these contractions and the ultrasound endometrial character, the character of the follicles, hormonal values, and to the occurrence of pregnancy (Ijiland et al. 1999). The authors found that, in contrast to non-pregnant patients, pregnant patients have a significantly different frequency of occurrence of the various wave types on the day of hCG stimulation. In addition, they found that there was a switch of the predominant wave direction from fundo-cervical to cervico-fundal, and they also showed that a premature wave direction switch was associated with a low pregnancy rate. The authors proposed that ovarian monitoring in IVF cycles should be supplemented by uterine monitoring for the endometrial wave-like activity, and they suggested that uterine endometrial wave-like activity patterns reflect the receptivity of the uterus (Ijiland et al. 1999). The

authors concluded that endometrial wave-like activity patterns appeared to be more promising than the thickness or texture of the uterus as an indicator of endometrial receptivity in IVF cycles, and they also hypothesised that endometrial thickness and texture may reflect the response of the endometrium to stimulation, while endometrial wave-like activity may reflect the integrated receptivity of the uterus as a whole (Ijiland et al. 1997).

A study of 209 infertile patients looked into the consequences of uterine junctional zone contraction on IVF-ET success rates (Fanchin et al. 1998b). The patients were exposed to the same level of ovarian stimulating drugs and they were chosen according to certain criteria with the aim of avoiding other factors which might affect the result, such as embryo quality and endometrial receptivity. Therefore, only patients with a morphologically normal uterus, who had at least 3 good quality embryos and an age of 38 years or below, were included. All patients underwent 5 minute transvaginal ultrasound scanning and their blood was collected for oestrogen and progesterone level monitoring. Just before the embryo transfer the type and the frequency of the junctional zone contractions was observed, see figure 5.4 (a) and (b). The authors found that there was a progressive increase in the prevalence of retrograde contractions (contractions from the cervix to the fundus), and a progressive decrease in the prevalence of antagonistic contractions (contractions from the fundus to the cervix). When the outcomes were analysed they found that patients with high frequency junctional zone contractions have a lower clinical and ongoing pregnancy and implantation rate, see figure 5.4(c), and table 5.1. There was also a significant negative correlation between plasma progesterone levels and uterine junctional zone contractions (Fanchin et al. 1998b). This study encouraged the researchers to search for the factors which exaggerate these uterine contractions and to try to minimise them.
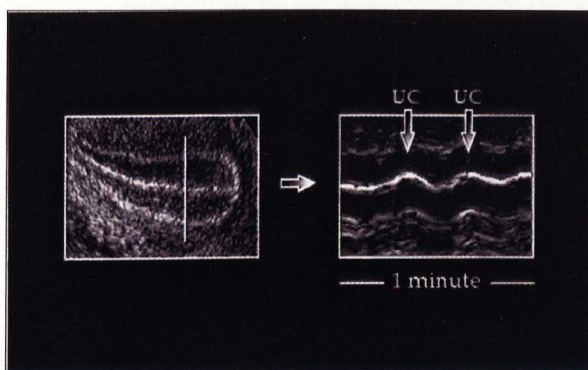
**Figure 5.4(a)** Computerized assessment of uterine contraction.
After determining the uterine section to be Analysed (left panel), Time-dependent changes in endo–myometrial interfaces corresponding to UC were assessed (right panel).
This figure is from Fanchin et al. (1998)
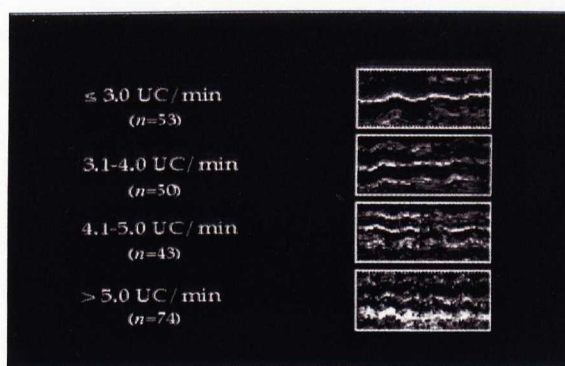


**Figure 5.4 (b)** Computerized assessment of uterine Contraction (UC) frequency
This figure is from Fanchin et al. (1998)
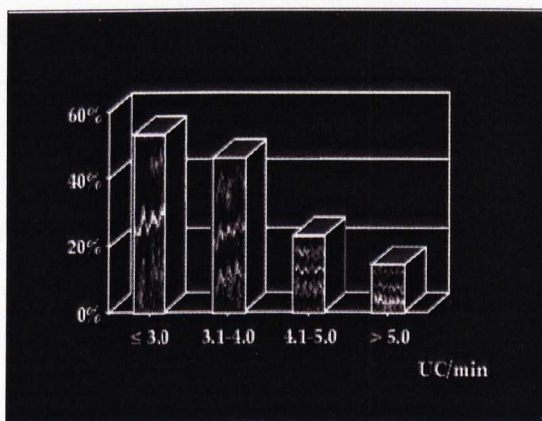


**Figure 5.4 (c)** Effect of the junctional zone contractions at the time of embryo transfer on the outcome of IVF-ET.
There is step wise decrease in the pregnancy rate with increase in the frequency of the junctional zone contractions.
This figure is from Fanchin et al. (1998)

**Table 5.1** Effect of the junctional zone contractions
at the time of embryo transfer on the outcome of IVF-ET.
[This table is from Fanchin et al. (1998, 2000a)]

| (4 days after HCG administration) | Pregnancy rate | Implantation rate |
|---|---|---|
| Uterine contraction >5 /min | 14% | 4% |
| Uterine contractions< 3 contractions / min | 53% | 21% |

## 5.10 Uterine junctional zone contractions at the time of blastocyst transfer:

Until recently the culture of embryos in the laboratory to the blastocyst stage was very difficult because the variety of culture media that were used to supply nutrients to the embryos were inadequate for extended embryo growth in the laboratory (Wilson et al. 2002). Therefore, many embryos died before they developed into blastocyst. Improved culture media are now available that sustain embryo growth in the laboratory for several days prior to implantation (Wilson et al. 2002). Many IVF centres now culture embryos to the later blastocyst stage before transferring them into a woman's uterus in an attempt to maintain or increase the pregnancy rate. Fewer of the later-stage embryos are transferred to reduce the risk of multiple gestations.

In animal studies evidence shows that during ovulation there is a release of prostaglandin and inflammatory factors from the ovary (Espey et al. 1992; and Kannisto et al. 1992). In human studies it was found that there is exaggeration of uterine contraction at the time of egg retrieval in hyper stimulated cycles when compared to natural cycles. It has been suggested that supra- physiological levels of oestradiol due to hyperstimulation and brief exposure to progesterone may be the underlying cause for this exaggeration of the junctional zone contraction in the IVF cycles (Lesny et al. 1998). These contractions were not noted at the time of hCG administration or immediately before oocyte retrieval. The exaggeration of junctional zone contraction following difficult embryo transfer (Lesny et al. 1998a) or the application of tenaculum (Lesny et al. 1999) have been suggested as the underlying cause though the outcomes may be due to local factors or neural stimulation that may be responsible for these uterine contractions (Lesny et al. 1998; 1999).

Research has found that high-frequency uterine contractions at the time of non-cavitating embryo transfer adversely influence IVF–embryo transfer outcome (Fanchin et al. 1998). The authors assessed uterine contractility in 43 IVF–embryo transfer candidates on the day of (hCG) administration, 4 days after hCG (non-cavitating embryo transfer), and 7 days after hCG (blastocyst transfers). The authors performed 2 minute ultrasound scans for the sagittal plane of the uterus; this was digitized with a computerized system for assessment of uterine contraction frequency. Results showed that there is significant decrease in uterine contraction frequency observed from the day of hCG ($4.4 \pm 0.2$ contractions/min) to ($3.5 + 0.2$ contractions/min) 4 days after hCG, with more decrease at 7 days after hCG ($1.5 \pm 0.2$ contractions/min; $P < 0.001$), see figure (5.5). They concluded that during the luteal phase of ovarian stimulation uterine contractility decreases progressively, and reaches a nearly quiescent status 7 days after hCG administration, at the time of blastocyst transfers. It is possible that such uterine relaxation assists blastocyst implantation and improves the success rate of IVF (Fanchin et al. 2001).
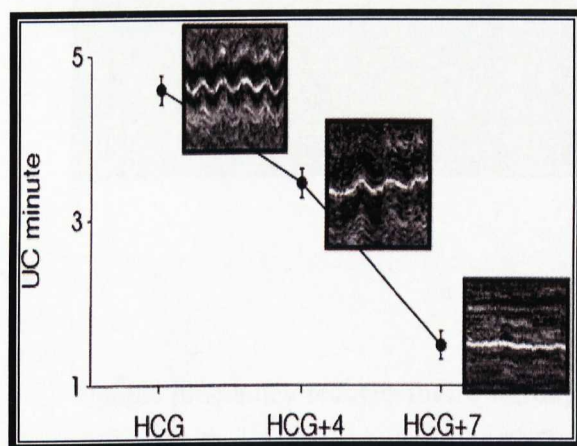


**Figure 5.5** Uterine junctional zone contractions on the day of blastocyst transfer showing a progressive decrease in the frequency of the junctional zone contraction from the day of hCG administration ($4 \pm 0.2$), ($3.5 \pm 0.2$) at hCG+4 days, to ($1.5 \pm 0.2$) at day 7 in the IVF cycle. This figure is from Fanchin et al. (2001)

## 5.11 The relation between ectopic pregnancy and uterine junctional zone contraction:

The first reference to ectopic pregnancy appears in the writings of the famous Arabic physician Abulcasis (Abdul Qasim Al Zahrawi 936 – 1013) (Lurie 1992). Abulcasis in his book (Al tasreef) described a patient who had abdominal swelling that underwent suppuration and drainage (Lurie et al. 1992). The first successful salpingectomy was reported by Lawson Tait who described it as the definitive treatment for tubal pregnancy (Tait 1884).

There is a high frequency of ectopic pregnancy after IVF or GIFT and the first clinical pregnancy after IVF was ectopic (Steptoe et al. 1976).



**Figure 5.6** Ectopic pregnancy. This photo is from advanced Fertility centre of Chicago, (2006) ©://www.advancedfertility.com/ectopic.htm) Accessed on 11/4/06 at 12.13pm

Ectopic pregnancy reduces future fertility and it is one of the leading causes of maternal mortality. It is also extremely distressing to many patients after their major efforts required to establish pregnancy (Edwards and Brody 1995).

The incidence of ectopic pregnancy after IVF is 2-5 % and it has been suggested that the underlying cause that could explain this high rate of ectopic pregnancy following ART, may be due to many patients who seek assisted conception having a defect in the fallopian tube (Marcus and Brinsden 1995). Further studies have been carried out by researchers trying to reduce this high rate of ectopic pregnancy (Lesny et al. 1999b; Amin and Sunny 2003). Research has shown that ectopic pregnancy was 3.9 times more closely associated with difficult embryo transfer procedures than with easy

ones (Lesny et al. 1999b). The authors put forward the suggestion that junctional zone contraction may be the underlying cause for this high rate of ectopic pregnancy. It was also shown that junctional zone contractions decrease with increasing time after oocyte retrieval (Lesny et al. 1998a). Other researchers found that there is a significant reduction in cervico-fundal contractions at day 7 after hCG administration compared with day 0 and day 4 after hCG administration Fanchin et al. (2001). Depending on the above finding, they suggested that the incidence of ectopic pregnancy might be reduced if the embryos were transferred at day 5 (blastocyst stage) (Fanchin et al. 2001).

Amin and Sunny (2003) compared the incidence of ectopic pregnancy between day 2 or day 3 embryo transfer versus day 5 embryo transfer,. They found that there was (3.5%) ectopic pregnancy in the first group and (3.9 %) ectopic pregnancy in the second group, and the difference between the two groups was not statistically significant. The authors suggested that factors other than the date of transfer predisposed to ectopic pregnancy after IVF treatment. Compared to day 2 or day 3 embryo transfer (early cleavage stage), blastocyst culture and transfer represent an effective means of eliminating high order multiple pregnancy. Implantation rate after extended blastocyst culture and transfer was found to be 32.4% and it is therefore significantly higher than that obtained after the transfer of a cleavage stage embryo (23.3%) (Wilson et al. 2002).

## 5.12 Uterine hyperperistalsis may be the cause of endometriosis:

Endometriosis is a common condition characterised by the presence of endometrial glands and tissues outside the endometrial cavity. It can be a debilitating disease for some women who experience ongoing pain or it may be asymptomatic and diagnosed only during infertility work up. Around one third of infertile women have endometriosis and it is not yet clear how the endometriosis affect the fertility (Koninckx et al. 1991).

Retrograde menstruation (Sampson 1927) is one of the theories, which gained widest acceptance though there are many other theories including coelomic metaplasia and mullerian remnant (Nissolle and Donnez 1997). Subsequently it was found that retrograde menstruation is a physiological phenomenon and it is observed in nearly all

menstruating women with patent tubes (Bartosik et al. 1986). However, it is still not understood why only 10-15% of those patients develop endometriosis, which is the incidence of endometriosis in the population (Nissolle and Donnez 1997).

Research has found that the defect is in the eutopic endometrium and the researchers explained this through the theory that altered endometrium has a higher potential of implantation and growth in the ectopic location (Wingfield et al. 1995).

Another study measured the transport of inert particles in 2 groups of patients (Leyendecker et al. 1996). In one group, the patients had endometriosis and the other group were endometriosis free. The authors found that uterine peristalsis is increased with nearly double the frequency of peristaltic waves during the early follicular, mid-follicular and mid-luteal phases of the cycle in comparison with healthy women. The authors also found that the inert particles are transported beyond the confines of physiological passive sperm transport and the amount of radioactivity measured in this group during the early follicular phase is increased 3 fold over the corresponding value for healthy women in the mid-follicular phase (Leyendecker et al. 1996).

Several studies have demonstrated that in patients with endometriosis, there is impairment of sperm transport and they concluded that the defect is primarily located at the level of the uterus (Leyendecker et al. 1996; and Kunz et al. 1996). Other studies used a variety of molecular and protein techniques and demonstrated that both endometriotic lesions and eutopic endometrium from women with endometriosis contain transcripts for P450 aromatase (Noble et al. 1997; Kitawaki et al. 1997). It has also been found that this enzyme acts in the conversion of C19 steroids (androgens like androstenedione and testosterone) to oestrogens (oestrogen and oestradiol) without changing the respective serum levels (Takahashi et al. 1989). These changes were found in the endometrium of patients with endometriosis but not the normal endometrium from the controls. The authors therefore concluded that this locally aromatase expression by eutopic endometrium may be related to the capability of these tissues to implant on peritoneal surfaces (Noble et al. 1997; and Kitawaki et al. 1997).

Transvaginal ultrasound for patients with endometriosis when compared with non-endometriotic patients has shown that there is a significant increase in the thickness of the junctional zone in patients with endometriosis as compared with the

control group (Kunz et al. 1998a). In addition, the authors found that there is no correlation between the thickness and the stage of endometriosis. Furthermore, they explained that both increased endometrial oestrogen concentration and expansion of the junctional zone have fundamental effects on junctional zone contractions (Kunz et al. 1998a). It has been found that locally increased oestrogen levels inevitably up-regulate the endometrial oxytocin mRNA (Zingg et al. 1995). This increased oxytocin level results in increased peristaltic movement of the circular muscle of the myometrium "junctional zone" (Kunz et al. 1998a). These contractions will lead to an increase in the intrauterine pressure (Bulletti et al. 1997) and increased trans-tubal seeding of the endometrium and finally infertility by impairment of uterine mechanism of rapid sperm transport (Kunz et al. 1996; and Leyendecker et al. 1996).

## 5.13 Is it possible to reduce the uterine contractions after embryo transfer by medications?

### 5.13.1. The use of vaginal progesterone:

Despite the advances in the technique of IVF-ET the rate of achieving pregnancy is still not justified. Factors which could improve the success rate have been analysed intensively and one of these factors was reduction of junctional zone contraction to avoid uterine contractions in the non-pregnant women. This was assessed previously by the use of an invasive method by insertion of a pressure probe in the uterine cavity (Henry and Browne 1943; Martinez et al. 1973). These invasive methods limit the study of the role of these contractions in human reproduction. Now, the advent of imaging techniques permits a rapid and non invasive detection of these contractions (Abramowitz and Archer 1990; Lyons et al. 1991). The role of uterine junctional zone contraction before and after embryo transfer was studied; the negative effect of high frequency contraction after embryo transfer on the success rate was reported( Fanchin et al. 1998b).

A study was done to investigate the effect of medications in the early luteal phase on the junctional zone contraction (Fanchin et al. 2001) studied the effect of early luteal phase support with progesterone gel (Crinone 8%, Ares –Serono S.A. Geneva,, Switzerland) in their study, they did transvaginal ultrasound scan for 2 minutes in 84 infertile women on the day of hCG, At the same time oestrogen and progesterone level

were estimated. The women were divided into 2 groups. In the first group, vaginal progesterone was started on the day of oocyte retrieval, while in the second group vaginal progesterone started on the evening of the embryo transfer. The scan was repeated for them just before the embryo transfer. It was found that despite the high level of systemic progesterone on the day of hCG administration, the level of the junctional zone contractions was high in the second group, while in first group, who had the vaginal progesterone on the day of oocyte retrieval, the level of contractions was reduced. It was concluded that vaginal route administration of progesterone is effective in reducing the junctional zone contractions on the day of ET as compared with the day of hCG, and it was suggested that this uterine relaxation before ET might avoid displacement of the embryo and improve the outcome (Fanchin et al. 2001). Other authors they found that decrease in uterine contraction frequency reached statistical significance on the third day of exposure to Progesterone without differences between the three dose groups of Crinone containing 45, 90, and 180 mg of Progesterone per application respectively (Ayoubi et al. 2001).

## 5.13.2. The use of antiprostaglandin and smooth muscle relaxant:

Prostaglandin was first described by the American gynaecologists Kurzok and Lieb in 1930 (Kurzok and Lieb 1930), they described the stimulatory effect of seminal fluid on human uterine muscle. Prostaglandins are unsaturated carboxylic acids synthesised from essential fatty acids (arachidonic acid) through the cyclooxygenase path way (Samuelsson 2003). Embrey (1971) found that prostaglandin (PGE2 and PGF2$\alpha$) stimulate myometrial contraction. Robins et al. (1975) suggested that prostaglandin is not only uterotonin but also uterotropin.

Wood (1981) found that the use of anti prostaglandin did not show any beneficial effect on the pregnancy rate. A similar result was obtained by Diedrich et al (1989). Shaker (1993) tried to reduce junctional zone contractions at the time of embryo transfer by the use of smooth muscle relaxant (Glyceryl nitrate); they found that the use of muscle relaxant at the time of embryo transfer did not affect the pregnancy rate.

### 5.13.3    Effect of nitric oxide on the contractility of the myometrium:

Nitric Oxide (NO) is an inhibitory neurotransmitter released from the peripheral neurons; it is derived from L-arginin, which is one of the 20 most common amino acid, and its 1 of 10 essential amino acid (Pickard et al. 1991). NO hyperpolarizes the smooth muscle and inhibits its contraction, and it is synthesised from catalytic oxidation of L-arginin by the action of the NO synthase enzyme (Moncada et al. 1991).

NO plays a crucial rule in many biological activities, in the process of reproduction and fertilization, NO is responsible for penile erection, female ovulation, menstruation, implantation, pregnancy maintenance, labour and delivery (Yallampalli et al. 1998; and Battaglia et al. 2002), the acrosome at the tip of the sperm head activates its NO synthase when it enters the egg. The release of NO in the egg is essential to block the entry of other sperms into the egg and it helps in the orientation of the pronuclei for fusion (Yallampalli et al. 1998).

In the human, the presence of NO- cGMP dependent pathway was first studied in the pregnant uterus (Yallampalli et al. 1993), they showed that the addition of L-arginin to the uterine muscle strip causes substantial relaxation in the tissue in addition they showed also that L-arginin causes dose dependent relaxation in the preterm pregnant uterus, further more they found that NO decreased during spontaneous delivery, and they explained that L arginin – Nitric Oxide system may be responsible for the quiescent state of the uterus during pregnancy, and diminished responsiveness of the uterine muscle to the NO at term, may lead to increase uterine contractions and initiation of labour (Yallampalli et al. 1993).

Evidence demonstrates that there is negative effect of uterine contraction on the pregnancy rate (Fanchin et al. 1998) and other studies show the positive effects of NO on the pregnancy rate in poor responder patient (Battaglia et al. 1999; Hoffman et al (2003). They suggest further experiments on human myometrium for better understanding of the modulation of myometrial contractility, before the use of NO in the IVF patient (Hoffman et al. 2003).

# CHAPTER SIX

## A prospective Study of Uterine Junctional Zone Contractions

### 6.1 Aim of the study:

Previous chapters of this thesis have presented data that identify embryo transfer as the point during IVF/ET, which might be amenable to improvement. In chapter five, I discussed the technique of measuring uterine junctional zone contractions as a possible means of assessing the technique of embryo transfer. The aim of this study was to assess the relationship between the frequency of uterine junctional zone contractions after embryo transfer and the outcome of IVF-ET.

### 6.2 Type of study:

This was a prospective observational study.

### 6.3 Patient characteristics:

113 patients who were having IVF-ET in the Hull IVF unit were invited to participate in the study and 50 agreed. All patients were counselled and participated on a voluntary basis and gave written consent. The research project was approved by the Hull and East Riding Local Research Ethics Committee.

### 6.4 Method:

All patients were given an information sheet on the day of egg retrieval, so that they could read about the aims of our research for the next two days before they had their ET. Their consent was then requested on the day of ET.

All the patients in the study had the same long protocol combining both complete pituitary desensitization with a GnRH agonist and ovarian hyperstimulation as mentioned in chapter two. In addition, they had a mock embryo transfer at the time of down regulation to measure the uterine length and to determine the most appropriate catheter type. Oocyte retrieval was performed under i.v. sedation using Midazolam and Alfantanil. Embryo transfer was done within 2-3 days after egg retrieval. All patients had additional ultrasound scans before and after embryo transfer as described below. Each embryo transfer procedure was carried out in the lithotomy position without anaesthesia. A speculum was used to fully expose the cervix and the use of a tenaculum avoided. During embryo transfer, the catheter tip was placed 1cm from the uterine fundus.

Statistical analysis was with the use of the SPSS statistic program of social science. Chi square test was used and a p value of <0.05 regarded as statistically significant.

## 6.5 *Imaging technique:*

A transvaginal ultrasound scan (Voluson 730 Pro, GE Healthcare, Pollards Wood, UK) recorded the images in the mid-sagittal plane of the uterus for 2 minutes before and 2 minutes after each embryo transfer. Every effort was made to keep the ultrasound transducer as still as possible so that the only movements recorded would be those of the uterine contractions. These images were stored digitally on a Dell laptop computer using Adobe Premiere Pro software and converted to x 5 normal speed. The frequency of contractions was determined by visual observation of the speeded up images. Uterine contraction frequency before embryo transfer was compared with the frequency after embryo transfer in order to see if the technique used for ET stimulated the uterus. In addition, the outcome of each IVF-ET cycle was compared with the frequency of junctional zone contractions, both before and particularly after ET.

# 6.6 Results:

Unfortunately, more than half of our patients refused to participate in the study and to have the additional ultrasound scans, particularly those who had had previous failed IVF-ET cycles. Some said they were concerned that the vaginal probe might cause displacement of the embryos.

In the study, our patient ages ranged between 25-44yrs with a mean age of 38yrs. All the women had transfer of two good quality embryos. The cause of their infertility is shown in table (6.1).

**Table 6.1** Aetiology of infertility of all patients who had ultrasound at the time of embryo transfer in the prospective study

| Cause | No. |
|---|---|
| PCOS | 1 |
| Endometriosis | 1 |
| Male factor | 24 |
| Tubal | 9 |
| Recipients of egg donation | 3 |
| Unexplained | 7 |
| Anovulation | 2 |
| Male factor and PCOS | 1 |
| Male factor and endometriosis | 1 |
| Donor sperm | 1 |

In this prospective study, we did not find any change in the frequency of junctional zone contractions after embryo transfer. This may be because of the near-perfect technique of the person performing the embryo transfer procedure (trying to avoid any excessive manipulation of the cervix and uterus). In all the cases both the characteristics and the frequency of waves after embryo transfer remained the same as before embryo transfer. Easy embryo transfer was possible in all cases randomized to this study group, probably, at least in part, because of the information gained at the careful mock embryo transfers.

In our study, we had 50 patients who agreed to have 2 minutes ultrasound before and after embryo transfer, 11 of them had a positive pregnancy test 2 weeks later. We found that there were 214 total contractions before embryo transfer, and 215 total contractions after embryo transfer, and we can see that the number of contractions is almost the same before and after embryo transfer, this means that the embryo transfer procedure is not creating more contractions after the procedure. Analysing the contractions before and after embryo transfer according to their type we found that there were 25 (11.68%) fundocervical contraction (FC), 124 (57.94%) random contractions (RC) and 74 (34.57%) cervico fundal contractions (CF) before embryo transfer. While the contractions after embryo transfer, were 33 (15.34%) FC, 113 (52.5%) RC, and 69 (32%) CF, see table 6.2. We can see that the frequency of the contractions is almost identical before and after embryo transfer, and the type of contractions is mainly random and Cervico-fundal contractions.

**Table 6.2** Frequency and type of junctional zone contractions (JZC) before and after embryo transfer

|  | Total | Fundo-cervical contractions | Random contractions | Cervico fundal contractions |
|---|---|---|---|---|
| JZC Before ET | 214 | 25(11.68%) | 124(57.94%) | 74 (34.57%) |
| JZC after ET | 215 | 33(15.34%) | 113(52.5%) | 69 (32%) |

If we compare the difference in the outcome between those who have <5 contractions per 2 minutes after ET, and those who have > 5 contractions per 2 minutes after ET, we find that 37 patients had < 5 JZC/2 minutes of which 11 had a positive pregnancy test after 2 weeks, making the pregnancy rate 29.7%. 13 patients had >5 JZC/2 minutes and all of them had a negative pregnancy test 2 weeks later making the pregnancy rate for this group 0%, P = 0.026 (<0.05). Hence there is a statistically highly significant difference in the outcome between patients who had more than 5 contractions and those who had less than 5 contractions over a 2 minute period after

embryo transfer. Junctional zone contractions are associated with a poorer outcome of IVF-ET, see table (6.3).

**Table 6.3** Difference in the pregnancy rate between patients who had <5 contractions in 2 mins after embryo transfer as compared with patients who had > 5 contractions in 2 mains after embryo transfer. Difference was checked with Chi square test

|  | < 5 JZC/2min after ET | >5JZC/2 min after ET | P value |
|---|---|---|---|
| N | 37 | 13 | |
| Pregnancies | 11 | 0 | |
| Pregnancy rate /ET | 29.7% | 0% | 0.026(<0.05) |

Ultrasound is known to have the potential to heat tissue and even though safety levels were maintained during this study, it is possible that the extended ultrasound examinations might have acted on either the uterus or the embryos so as to reduce the pregnancy rate. In order to check whether the prolonged ultrasound examination was harmful for each of the 50 patients in the study the result of the next treated patient not in the study was recorded. 12 patients out of this control group of 50 become pregnant, making the pregnancy rate 24%, which is not significantly different from the 22% seen in our study group [P >0.629]. Hence, ultrasound has no detrimental effect on the outcome of IVF-ET, see table (6.4).

**Table 6.4** Effect of extended ultrasound scanning on the outcome of IVF-ET.

|  | Study patients | Controls | P value |
|---|---|---|---|
| Pregnancy rate/ET | 11/50 22% | 12/50 24% | 0.629 |

## 6.7 Discussion:

The present study was conducted to investigate the possible effect of Junctional Zone Contractions (JZC) as visualized by ultrasound at the time of embryo transfer on the outcome of IVF. We observed poorer rates of pregnancy in patients displaying higher JZC frequency in comparison with those presenting lower JZC frequency.

Brinholz (1984) originally reported the visualization of JZC in the non pregnant women. With the use of transabdominal ultrasound, uterine contractility was not seen in 27% of cases. More recently, transvaginal ultrasound was used to visualize JZC during the menstrual cycle, and it was found that the frequency and the amplitude of these contractions increase progressively during the follicular phase to a maximum activity at mid-cycle before declining throughout the luteal phase (Abramowicz and Archer 1990). De Vries et al. (1990) found that there is a rhythmic contraction of the inner third of the myometrium in pregnant, nonpregnant, and postmenopausal women, and the majority of women showed Cervico-fundal contractions. While in menstruating women and cases of abortion, the contractions were antegrade (Fundo-cervical). They concluded that these retrograde contractions of the inner third of the myometrium might be important in sperm transport and for the conservation of early pregnancies within the uterine cavity.

The Physiological role of JZC at mid-cycle is probably to promote sperm transport through the uterine cavity to the Fallopian tubes (Lyons et al. 1991; Kunz et al. 1996). In the luteal phase, the progressive reduction in uterine contractility may be necessary for the contact between blastocysts and endometrium and may therefore assist implantation (Abramowicz and Archer 1990; Lyons et al. 1991). The fundocervical contractions observed during the menstrual phase probably help to get rid of the menstrual debris. Knutzen et al. (1992) suggested that the considerable mechanical stimulation of the uterus as a result of the embryo transfer procedure might further stimulate the myometrial activity.

Oike et al. (1990) found that there is a positive correlation between the frequency of peristaltic movements and the oestradiol level in the proliferative phase, and oestradiol level in the secretory stage of the cycle. Same authors found that the detection rate was lowered significantly when the serum progesterone level was more than 3.8 ng/ml. Other studies described uterine junctional zone (JZ) contractions in both natural cycles (Ijiland et al. 1996; 1997b), and assisted reproduction cycles (Lesny et al. 1998b).

In our study, we found that random contractions were the main type of contraction (57.94%) seen at the time of embryo transfer. Our results are consistent with the result of a previous study by Lesny and co workers who found that Cervico-fundal contractions were present in 3/14 (21.42%) of cases, but the contractions were short and did not involved the whole length of endometrium. In addition, they noted that opposing contraction were present in 7/14 (50%) of patients and random waves were present in 11/14 (78.57%) of patients (Lesny et al 1998a).

Ijiland et al showed that there was a lower frequency of these contractions throughout natural cycles and that different contraction characteristics are associated with a higher pregnancy rate (Ijiland et al. 1997a, 1999). Similar results were achieved by Fanchin and co workers in stimulated cycles. Fanchin et al. (1998a) who showed that increased JZ contractions at the time of embryo transfer are associated with a lower pregnancy rate. The result of our prospective study is consistent with the result of Fanchin et al 1998, as we also found that exaggerated junctional zone contractions at the time of embryo transfer have detrimental effect on the pregnancy rate after IVF-ET. Different results were achieved by Woolcott and Stanger (1997), as they reported an improved IVF-ET outcome when uterine contractile activity was present just after embryo transfer. Lesny et al. (1998b) showed that difficult embryo transfers in oocyte donors are associated with stimulation of the junctional zone contractions and can cause displacement of the embryos towards the cervix and into the fallopian tubes. In addition, they demonstrated that increased junctional zone contractility after a difficult embryo transfer could be seen as late as 45 min after the procedure and they concluded that there is no point in waiting for the release of embryos from the catheter or keeping the

catheter 'in situ' for 60 seconds after embryo transfer as recommended by Al-Shawaf et al. (1993b) and Wisanto et al.(1989). In our study, all our embryo transfer procedures were easy and we did not have difficult embryo transfer i.e. in all our 50 cases embryo transfer catheter was not changed, a tenaculum was not used and all the procedures were very smooth and performed with same catheter that was chosen on the day of mock embryo transfer. For this reason, we did not have the chance to see the difference in the character and the frequency of contraction before and after difficult embryo transfer, and whether these contractions become exaggerated or not.

## *6.8 Conclusions:*

We conclude that uterine junctional zone contractions are inherently present whatever precautions are taken to avoid uterine stimulation during embryo transfer. Furthermore, we found that exaggerated uterine contractility has a negative effect on the outcome of IVF-ET. Previous studies support the view that this may cause embryo displacement and consequently implantation failure. For this reason, it is reasonable to suggest that several precautions should be taken to avoid the initiation of uterine contractility. This can be achieved by using soft catheters, gentle manipulation, avoiding touching the fundus, avoiding using a tenaculum, and considering ZIFT rather than TMET in cases where transcervical transfer is not possible and at least one fallopian tube is patent. Moreover, in the future we may find a drug that can be used as a standard treatment to reduce the frequency of these contractions in order to improve the outcome of IVF-ET.

# CHAPTER SEVEN

## 7.1 Recommendations for embryo transfer

Embryo transfer is the most critical step in the IVF process and as such extra care and time should be devoted to ensuring the highest possible chance of a successful transfer. This final hurdle in assisted reproduction will determine the result of a sustained period of effort and concern on the part of all those involved. Everything from ovulation induction and ovum retrieval, to insemination and embryo culture and the work of embryologists to maintain the viability of embryos is futile if a traumatic and subsequently unsuccessful embryo transfer follows. Englert et al. (1986) reported a 33.3% pregnancy rate with transfers rated "excellent", while "bad" transfers yielded only a 10.5% pregnancy rate.

## 7.1.1 Mock embryo transfer

In order to ensure the highest chance of a successful embryo transfer the following suggestions may be considered. Evaluation of the uterine cavity before the IVF cycle is important and mock embryo transfer before the IVF cycle begins is one of the measures that enable us to perform this assessment and ensure the proper placement of the embryos. Mock embryo transfer allows us to evaluate the length and direction of the uterine cavity as well as the cervical canal and to choose the most suitable catheter for the embryo transfer. It also helps to reveal any unanticipated difficulties in entering the uterine cavity, such as a pinpoint external os, the presence of a cervical polyp or fibroids. It may also show anatomical distortion of the cervix from previous surgery or due to congenital anomalies. Another way of evaluating the uterine cavity is through the use of ultrasonography (US). This gives precise information about the length of the uterine cavity, the length of the cervical canal and the degree of the cervical angulation in relation to the uterine cavity. Ultrasonography is also very important for diagnosing any fibroids that may be distorting the endometrial cavity or the cervical canal.

## *7.1.2 Uterine junctional zone contractions*

Initiation of uterine contractions may produce worse outcomes and as such, several precautions should be taken to avoid this. Gentle manipulation should always be the rule, even when introducing the speculum to avoid unnecessary pressure on the cervix. It has been observed that the use of tissue forceps to hold the cervix can trigger uterine contractions (Lesny et al. 1999a) therefore, holding the cervix by a tenaculum should be completely avoided except in rare cases.

## *7.1.3 The transfer catheter*

The embryo transfer catheter should be soft and smooth enough to avoid any trauma to the endometrium or to the endocervix and flexible enough to find its way into the uterine cavity. Several studies have compared different kinds of catheters for embryo transfer and have demonstrated that soft catheters produce the best results in terms of pregnancy rates (Wisanto et al. 1989; Mansour et al. 1994; Cohen 1998; and Wood et al. 2000). In addition, the outer rigid sheath should be minimally used with the aim of just stopping short of the internal os. The stimulus of the transfer catheter passing through the internal cervical os can also initiate contractions, which are probably mediated by the release of prostaglandins (Fraser 1992). Due to this risk of contractions in humans, it is advisable to perform the embryo transfer without manipulation of the cervix (Dorn et al. 1999; Lesny et al. 1999a). If cervical stenosis is diagnosed, it is advisable to perform cervical dilatation before ovarian stimulation.

At the time of embryo transfer, it is essential to be absolutely sure that the embryo transfer catheter has passed the internal os and entered the uterine cavity. Soft catheters can sometimes be misleading as they can curve inside the cervical canal. Some authors suggest waiting before withdrawal of the catheter so that the uterus can stabilize (Wisanto et al. 1989; and Al-Shawaf et al. 1993a). However, other authors achieved good results by withdrawing the catheter immediately after the transfer (Zech et al. 1997).

## 7.1.4 Embryo quality

Embryo quality is a critical parameter in human IVF-ET, and one of the most difficult aspects of assisted reproductive technology (ART) is the determination of which embryos are most suitable for transfer into the uterus. Embryos exhibiting irregular shaped blastomeres and severe fragmentation are considered poor quality embryos and show a higher incidence of chromosomal abnormalities 62% compared to embryos of good quality 22.2% (Almeida and Bolton 1996). Fragmentation has been shown to be correlated to chromosomal mosaicism (Munne and Cohen 1998). In addition, slow cleaving and rapidly cleaving embryos show a higher incidence of chromosomal aneuploidy (Magli et al. 1998).

Evaluation of embryo quality determines which and how many embryos will be transferred and as pregnancy rates are directly related to the number and the quality of the transferred embryos, see chapter (2), tables 2.11(a), (2.14), and figures (2.9), (2.11) this is obviously of central importance. Embryos are usually classified according to the presence of cytoplasmic fragmentation, regularity of the blastomeres and cleavage rate (Staessen et al. 1992). However, a fragmented embryo can produce a normal pregnancy, though this occurs less frequently than with non-fragmented embryos (Veeck 1987). Therefore, it is suggested that poor quality embryos can be an inherent feature in human fertility.

## 7.1.5 Cleavage rate

In our retrospective study, we confirmed that embryo cleavage rate is another important factor relating to embryo viability. Embryos with slower cleavage rates have lower rates of implantation. This study shows that in a comparison between the transfer of embryos at the 2 or 4 cells stage versus embryos at the 6 or 8 cells stage, the high cleavage rate embryos found in the 6 or 8 cells stages yielded higher pregnancy and implantation rates, for a similar degree of fragmentation when compared to 2 or 4 cell embryos, see chapter (2), table 2.18(a),2.18(b) and figures (2.12), (2.13). The presence of fragmentation has been shown to reduce the

implantation rate (Staessen et al. 1992) and equally cleavage rate is another important factor reflecting the embryo viability (Edwards and Brody 1995). However, this means that choosing the right embryo becomes a crucial issue in order to maintain a good chance of pregnancy.

## 7.1.6 Number of embryos transferred

Several programs have limited the number of embryos transferred to two due to the increased incidence of multiple gestations after IVF and embryo transfer (HFEA 2004); however, reducing the number of embryos for transfer will not entirely eliminate multiple gestations. Monozygotic twinning has been reported to be higher following assisted reproduction (Edwards et al. 1986; Wenstrom et al 1993). The exact mechanism of monozygotic twin formation in ART is unknown; it has been ascribed to ovulation induction (Derom et al. 1987) or ART culture conditions (Edwards et al. 1986). Limiting the number of embryos transferred has lead to a dramatic decrease in the number of triplets but the risk of twin gestation is still high at around 25% (Devreker et al. 1999). To reduce the risk of twin gestation the transfer of only one embryo may be a good alternative.

## 7.1.7 Maternal age

Maternal age is another critical parameter in human fertility. In our retrospective study, we confirmed the effect of maternal age on the outcome of IVF-ET, see chapter 2, table (2.4) and figure (2.4). With increasing maternal age pregnancy rates decrease due to the progressive depletion of the ovarian follicle store with a subsequent decrease in the number oocytes that can be collected and therefore a smaller number of embryos are available (Spandorfer et al. 1998). While embryo quality reflects embryo viability it has been shown that embryos that appear to be perfect can contain DNA abnormalities and that the frequency of these abnormality can increase with an increase in maternal or paternal age (Munne et al. 1995). This may explain the lower pregnancy rates observed in this study for older women when compared to younger women with similar embryo quality. We also found that infertility due to male factors is more closely correlated with poor quality

embryos. Transfer of embryos of a high quality will allow reduction of the number of embryos transferred therefore leading to a reduction in the risk of multiple gestations. As multiple gestations are a major issue in assisted reproduction programs risk should be avoided wherever possible. Therefore, the transfer policy should be modified to replace only one embryo of good quality in patients with a good prognosis i.e. those of a low age, especially with tubal causes of infertility. This policy will encourage the use of mild ovarian stimulation, which has been recommended (Edward et al. 1996). This may lead to avoidance of maximum collection of oocytes and embryos if we are going to use only one or two embryos. Blastocyst transfer may be a good solution but it requires the use of more appropriate culture media and still remains to be confirmed by a randomised study. Since the uterus may be a better milieu than current blastocyst media preparations, we should be cautious in assuming that failure to reach the blastocyst stage indicates that the embryo never had implantation potential. It is also important to note that between 2% and 40% of patients will have their embryo transfer cancelled because no embryos developed to day 5 (Alper et al. 2001). It may be pertinent to ask whether some of these patients would have had a reasonable chance of success if they underwent a transfer of their embryos at the cleavage stage.

## 7.1.8 Cryopreservation

Freezing programmes increase the chance of pregnancy per cycle; see chapter (2), table (2.9), and figure (2.7), with patients undergoing controlled hyperstimulation as an outpatient. In conclusion, the possibility of choosing embryos for transfer remains the main determinant of the outcome and prospective and randomised studies should improve the technique of embryo selection for each patient. From the previous points we can see that attention to the details of the technique of embryo transfer appear to be as important as the effort of the embryologist in the laboratory in the final success of IVF.

## 7.1.9 Future possibilities

In the future, we may learn more about junctional zone contractions and uterine relaxant drugs may become standard treatment at the time of embryo transfer.

Improving the catheter design may also increase the efficiency of the embryo transfer without damaging the uterine cavity.

We might find a new marker for endometrial receptivity that will enable us to better understand the conditions necessary for implantation. Along similar lines, pre-transfer endometrial sampling may become a standard to confirm that conditions are compatible with implantation.

Abstinence is commonly practised during infertility treatment and often recommended by the treating physician. Encouraging couples to engage in intercourse during IVF treatment might have psychological advantages in term of normalizing the conception process.

In the future, we may have an embryo marker that may determine which embryos have the highest developmental potential, and therefore allow the selection of fewer embryos (only those with the greatest potential for producing a pregnancy).

## 7.1.10 Day 2 versus day 3 transfer

Van Os et al. (1989) were the first to demonstrate that embryo transfer could be delayed from Day 2 to Day 3 without any detrimental effect on subsequent pregnancy rates. In addition, they found that a higher number of viable pregnancies were established following transfer of embryos on Day 3. In a later study, Dawson et al. (1995) demonstrated that although the pregnancy rate was not significantly different between Day 2 or Day 3 embryo transfer, the implantation rate was significantly higher on Day 3. Our results of the prospective study are consistent with the previous two studies; hence, it seems reasonable to suggest embryo transfer always on day 3 rather than day 2, see figure (2.14).

## 7.1.11 Operator experience

Nurses have a wide range of roles in the treatment of infertility. Their roles vary from primary assessment and referral of infertile couples (Saulsbery and Pohlaus 1992), to a specialized nursing role in infertility counselling (Jennings 1992), and moral support (Clapp 1985). In the Hull IVF Unit, as with many other IVF units in the UK, nurses have extended roles including counselling, moral support, treatment planning, dose adjustment, consulting, ultrasound monitoring, egg retrieval and embryo transfer. The satisfactory results obtained and the extensive experience without complications demonstrates that this role is effective. The availability of proper supervised training is an important issue here as it was shown in our study that a new trainee or nurse may take around one year or the experience of performing more than a hundred embryo transfer procedures before they can be regarded as well experienced, see chapter (2), table (2.23), and figure (2.17). There should also be a clearly defined limit of responsibility to avoid any medico-legal issues.

## 7.1.12 Hydrosalpinges

Patients with hydrosalpinx should be offered surgical treatment as early as possible after the diagnosis to avoid possible permanent damage to ovarian function or to the endometrium (Freeman et al. 1998). For those patients with documented endometrial fluid accumulation, freezing of the resulting embryos should be considered. This should be followed by surgical correction of the hydrosalpinx prior to transfer of frozen embryos, see chapter (2), table 2.26(a), 2.26(b), and figure (2.21).

## 7.1.13 Oocyte donation

The majority of studies suggest that oocyte aging is the main factor responsible for decreasing fertility with age. Oocyte donation was introduced in 1984 to treat premature ovarian failure. The original indications for oocyte donation were premature menopause, genetic disease and inaccessible ovaries; however with the passing of time, several other indications have been suggested. The increasing pregnancy rate from oocyte donation strongly supports this evidence and indications for oocyte donation include: resistant ovary syndrome, abnormal oocytes during IVF

treatment, surgical castration or following chemotherapy or radiotherapy, ovarian dysgenesis and in women with hereditary genetic disease (Anselmo et al. 1999; and Ahuja et al. 1999).

## 7.1.14 Timing

In all instances, the most critical component is the synchronization between the donor and the recipient for the appropriate timing of embryo transfer. The recipient must also have oestrogen and progesterone therapy to prime the endometrium prior to ET. The age of the woman producing the oocyte affects the chance of implantation of an embryo in a manner that appears to be independent of other factors contributing to the infertility of the couple. The obstetric profile of pregnancies following oocyte donation poses a special risk to the mother and to the fetus. In pregnancies following oocyte donation there is a high incidence of pre-eclampsia and intrauterine growth retardation. The negative influence of the increased maternal age may contribute to the above complications. Due to the relative short amount of time elapsed since the introduction of oocyte donation the effects on the long term physical and psychological development of these children is still unknown.

## 7.2 Primary aim of the thesis

In my thesis, my aim was to identify factors that might influence the outcome of IVF-ET treatment and which might be amenable to modification in order to improve IVF pregnancy rates. I realize that many factors can affect a couple's chance of achieving pregnancy and birth by using IVF-ET or IVF/ICSI and one of the most significant factors of these is maternal age. I found that with increasing maternal age the probability of ongoing pregnancy established by the use of IVF-ET decreased. Certain parameters like the antral follicle count can be used as a predictive for the success to identify if women over 40 years still have favourable prospect (Broekmans and Klinkert 2004). I also found that increasing male age can affect the outcome of IVF-ET. Dunson et al. (2002) reported that fecundity begins to decline in the late 20s for women and in the late 30s for men. They suggested that the effect of male age on fecundability can be minimized by timing intercourse to

the days with optimal cervical secretions. There are clear cut indication for ART and medical practitioners should refer infertile patients under their care that require IVF-ET or IVF/ ICSI to ART centres before age catches up with either the female or the male partner.

Generally, it is agreed that advances in the laboratory resulting in better quality embryos are perhaps the most significant reason why the success rate of IVF-ET has increased. Laboratory condition such as cleanliness, room lighting, odour, culture media, and incubators (i.e. temperature and $CO_2$ level) should be optimised in order to achieve maximum success. In my study I found that 4 cell embryos transferred on day two gave rise to significantly higher pregnancy and implantation rates as compared with early cleavage embryos, and similar result was found with 8 cell embryos transferred on day three as compared to slow cleaving embryos transferred on the same day. In addition, I found that selecting good quality embryo on the basis of morphology helps to significantly raise the pregnancy and implantation rates. This may encourage a change in policy to reduce the number of transferred embryos in good prognosis patients and reduce the chance of a multiple gestation as a result. This suggestion can be supported by our result which shows that the transfer of 3 fresh embryos give lower pregnancy rate but higher twin and triplet rates.

Cryopreservation has proven to be an extraordinarily useful procedure in assisted reproductive technology. It allows multiple embryo transfer from single egg retrieval; it avoids frequent exposure of the patients to the risk of OHSS, with acceptable pregnancy and implantation rates. Optimization of the technique of freezing, thawing, and choosing the optimum type of the cryoprotectant agent may improve the outcome of IVF-ET in the freezing cycles, but it is obviously of the utmost importance that these excellent results are not overshadowed by neglect of the ethical and legal problems of cryopreservation. Otherwise, the advantages of cryopreservation will be lost.

In my study, I tried to clarify the optimal date for embryo transfer. I found the outcome was better when embryo transfer was performed on day five or day three rather than day two, which means that delaying embryo transfer one day, may allow a more accurate selection of the most viable embryo. In addition, I found that blastocyst transfer carries a significantly higher pregnancy and implantation rates,

but the main problem is an increased embryo transfer cancellation rate. Optimization of the laboratory condition and culture media may reduce the cancellation rate and improve the IVF-ET outcome. Levitas et al. (2004) found that the ET cancellation rate was reduced significantly if the decision to proceed to blastocyst transfer was made on day 3 after oocyte retrieval, and they suggested doing blastocyst transfer in patients who failed to conceive in at least three IVF/ET cycles.

I found also that there is a difference in the outcome of IVF-ET according to the technique of embryo transfer, which is either TCET or TMET with a better outcome when the procedure is done transcervically even if this is difficult, and to consider ZIFT when TMET is indicated if at least one fallopian tube is patent. Some factors encouraged us to recommend ultrasound-assisted transfer, especially in previously difficult cases.

I found that there is a difference in the outcome according to the aetiology of infertility with the best outcome when the cause of infertility was tubal. I found that there is a difference in the outcome between treated and untreated hydrosalpinx, especially when there is an intrauterine fluid collection. Therefore, we should consider removal of the hydrosalpinx before the start of IVF cycles and freezing the embryos and postponed the embryo transfer procedure when intrauterine fluid accumulation was identified at the time of embryo transfer. I found that variation in the embryo transfer technique among different physicians has a profound impact on individual pregnancy rates in the same situation with a good outcome when nurses perform the embryo transfer procedure. We should consider leaving all straightforward embryo transfer procedures to be done by the nurse. This will bring a potential benefit for the couples by ensuring good continuity of care, which increases their confidence and reduces their stress, but there should be a clearly defined limit of responsibility and agreed protocols as well as availability of an appropriate supervised training program.

By studying the results of the egg-sharing programme, I showed that aging of the ovary is more important than aging of the uterus, and that pregnancy rates in egg recipients were almost the same as the pregnancy rate in egg sharers of the same age group. Abortion rates were higher and consequently live birth rates were lower with increasing age of recipient but the difference was not statistically significant. In addition, I found that there is no difference in outcome between egg sharers and

standard IVF patients, which means that egg sharing has no detrimental effect on the outcome for egg sharers, and this program can be used for a wide range of patients with conditions such as premature ovarian, primary ovarian failure or a history of genetic disease; but the utmost important point is not to neglect the legal and the ethical issues.

# 7.3 Second aim of the thesis

The retrospective nature of this study may be a limiting factor for my conclusions but the results may serve as a basis for further large randomised prospective studies. Therefore, my second aim was added in which I tried to identify the ways in which the quality of any modification might be judged with out the need of a large clinical trial. The only factor which was found to be amenable to modification and which might be relevant was the technique of embryo transfer. In order to meet the second aim, two methodologies were employed

## 7.3.1 Targeted literature review

Firstly, I performed a review of the published literature which showed that the embryo transfer technique is the critical phase of IVF. Successful completion of all other processes is in vain if embryos are damaged, misplaced or reflux into the cervix, vagina or fallopian tube. For optimization of pregnancy and implantation rates smooth catheters should be used and blood, mucus, bacterial contamination and trauma to the endometrium should be avoided. An ideal catheter design that does not cause any endometrial irritation or trauma at the time of embryo transfer may be found in the future.

## 7.3.2 Randomised prospective study

Secondly, I performed a prospective randomised study of junctional zone contractions to identify the effect of these contractions on the outcome of IVF-ET. I demonstrated that exaggerated junctional zone contractions have a detrimental effect

on the outcome of IVF-ET. This result is consistent with the study by Fanchin et al. (1998b), in which they concluded that uterine relaxation before ET is likely to improve IVF-ET outcome by avoiding the displacement of embryos from the uterine cavity. Lesny et al. (1998a) found Junctional zone activity throughout the IVF cycle to be more exaggerated when compared to the results reported from observations of the natural cycle but following a similar pattern. In a further study, Lesny et al. (1998b) found that an easy mock embryo transfer did not change endometrial mechanical activity, and demonstrated that Echovist remained in the upper part of the uterine cavity and was not dispersed after 45 min. In contrast, after a difficult mock embryo transfer strong junctional zone contractions, were seen to relocate intrauterine embryos and this activity seemed to depend on physical stimulation of the uterus. Furthermore, they concluded that junctional zone contractions could be implicated in cases of IVF/ET failure or ectopic gestation. Lesny et al (1999a) found that manipulation of the cervix with a tenaculum stimulated junctional zone contractions and they suggested avoiding the use of tenaculum at the time of embryo transfer. In a study published in 2001, Fanchin et al. found that vaginal progesterone administration starting on the day of oocyte retrieval produced a decrease in uterine contraction frequency on the day of ET as compared with preovulatory values. William et al. (2001) found that pregnancy rates were significantly decreased by initiating luteal-phase progesterone supplementation on day 6 after oocyte retrieval during in vitro fertilization cycles. Ayoubi et al. (2001) found that a decrease in uterine contraction frequency reached statistical significance on the third day of exposure to Progesterone without differences between three dose groups. Biervliet et al (2002) found an increase in the junctional zone contractions with TMET, and they concluded that the increase in junctional zone contractions after transmyometrial embryo transfer forms a theoretical objection to this procedure.

## *7.4 Suggestions for further study*

Further experiments on human myometrium for better understanding of the modulation of myometrial contractility are required.

The prospective study of uterine contractions described in this thesis has developed the methodology whereby different techniques for embryo transfer can

now be evaluated without the need for comparing pregnancy outcomes between large groups in order to obtain statistical significance.

In the future, a new medication may be found that will be able to suppress the exaggerated junctional zone contractions and hence could be used as a standard treatment at the time of embryo transfer to improve the outcome of IVF-ET.

The only current alternative to a difficult transcervical embryo transfer, TMET, is also associated with an increase in junctional zone contractions. We therefore suggest a prospective study to investigate the most effective method of embryo transfer in cases where a difficult transcervical embryo transfer is anticipated due to cervical factors.

# Reference:

Abdalla, H.I. (1996). A national oocyte donation society is needed. *Hum.Reprod.* Vol. **(11)**: pp. 2355-2356.

Aboulghar, M. Mansour, RT., and Serour, G.I. (1999). Management of long standing infertility. *Am.J. of Obstet. and Gynaecol.* Available on PDF (57 K) ,(Accessed on 10/05/06 at 8:32pm )

Abou-Setta, A.M., Al-Inany, H.G., Mansour, R.T., Serour, G.I., and Aboulghar, M.A. (2005). Soft versus firm embryo transfer catheters for assisted reproduction: a systematic review and meta-analysis. *Hum. Reprod.* Vol. **(20)**, no. **(11)**: pp. 3114-3121

Abramowitz, J.S., and Archer, D.F. (1990).Uterine endometrial peristalsis –a transvaginal ultrasound study. *Fertil. Steril.* Vol. **(54)** : pp. 451-454.

Abusheikha, N., Lass, A., Akagbosu, F., and Brinsden, P. (1999). How useful is cervical dilatation in patients with cervical stenosis who are participating in an in vitro fertilization–embryo transfer program? The Bourn Hall experience. *Fertil. Steril.* Vol. **(72)** : pp. 610–612.

Ahuja, K.K., Smith, W., Tucker, M., and Craft, I. (1985). Successful pregnancies from the transfer of pronucleate embryos in an outpatient in vitro fertilization program.
*Fertil. Steril.*; Vol. **(44)**, no. **(2)**: pp.181-184.

Ahuja, K.K., Simons, E.G., Fiamanya, W., Daiton, K., Amar, N.A., Kirkpatrick, P., Sharp, S., Arian-Schad, M., Seaton, A. and Watters, A.J. (1996). Egg-sharing in assisted conception: ethical and practical considerations. *Hum. Reprod.* Vol. **(11)**: pp. 1126–1131.

Ahuja, K.K., Simons, E.G., Mostyn, B.J., and Bowen-Simpkins, P. (1998). An assessment of the motives and morals of egg share donors: policy of "payments" to donors requires a fair review *Hum. Reprod.* Vol. **(13)**: pp. 2671–2678.

Ahuja, K.K., Simons, E.G., and Edwards, R.G. (1999). Morals and medical risks: conflicting notions underlying the recruitment of egg donors. *Hum. Reprod.* Vol. **(14)**: pp. 279-84.

Aitkin, R.J. (1990). *Br. Med. Bull.*;Vol. **(46)**: pp. 654-674. In Edwards, R.G. and Brody, S.A. (1995). Principles and practice of assisted human reproduction, chapter **(8)**: Oocyte growth maturation and fertilization: pp. 309.

Akerlund, M., Bengtsson, L.P., and Ulmtsen, U. (1978). Recording of myometrial activity in the non pregnant human uterus by a micro transducer catheter. *Obstet. Gynaecol.*; Vol. **(57)**: pp. 429-433.

Akman, M.A., Garcia, J.E., and Danewood, M. (1996). Hydrosalpinx affects the implantation of previously cryopreserved embryos. *Hum. Reprod.* Vol. **(11):** pp. 1013–1014.

Akman, M.A., Erden, H. F., and Bahceci, M. (2005). Endometrial fluid visualized through ultrasonography during ovarian stimulation in IVF cycles impairs the outcome in tubal factor, but not PCOS, patients. *Hum.Reprod.* Vol. **(20),** no. **(4):** pp. 904-909.

Al- Hussaini, T. (1992). Pregnancy following transvaginal gamete intra fallopian transfer (GIFT), zygote intra fallopian transfer (ZIFT), tubal embryo stage transfer (TEST) in an invitro fertilization program. *J. Assist. Reprod. In addition, Genet.* Vol. **(9):** pp. 402-404.

Allen, V.M., Wilson, R.D., and Cheung, A. (2006).Pregnancy outcome after assisted reproductive technology.*J Obstet Gynaecol Can.* Vol.**(28),** no.**(3):** pp.220-50.

Almeida, P.A., and Bolton, V.N. (1996). The relationship between chromosomal abnormality in the human preimplantation embryo and development in vitro. *Reproduction, Fertility, and Development*; Vol. **(8):** pp.235–241.

Almeida, P.A., and Bolton, V.N. (1998). Cytogenitic analysis of human preimplantation embryos following developmental arrest in vitro. *Reproduction, Fertility, and Development.* Vol. **(10):** pp.505–513.

Alper, M. M., Brinsden P., Fischer, R., and Wikland, M. (2001).To blastocyst or not to blastocyst? *Hum.Reprod.* Vol. **(16),** no. **(4):** pp. 617-619.

Alsalili, M., Yuzpe, A., and Tummon, I. (1995). Cumulative pregnancy rates and pregnancy outcome after in-vitro fertilization: .5000 cycles at one centre. *Hum. Reprod.* Vol. **(10):** pp. 470–474.

Al-Shawaf, T., Yang, D., and al-Magid, Y. (1993a).Ultrasonic monitoring during replacement of frozen/thawed embryos in natural and hormone replacement cycles. *Hum. Reprod.* Vol. **(8):** pp. 2068–2074.

Al-Shawaf, T., Dave, R., and Harper, J. (1993b). Transfer of embryo in to the uterus: How much do technical factors affect pregnancy rate? *J. Assist. Reprod. Genet.* Vol. **(10):** pp. 31-36.

Amarin, Z.O., and Obeidat, B.R. (2004). Bed rest versus free mobilization following embryo transfer: a prospective randomized study. *BJOG.* Vol. **(111),** no. **(11):**pp. 1273-1276.

American society for reproductive medicine revised classification of endometriosis (1997). *Fertil. Steril.* Vol. **(67):** pp. 817-821.

Amin, A. M., and Sunny, H. (2003).Ectopic pregnancy rates with day 3 versus day 5 embryo transfer: a retrospective analysis. *BMC Pregnancy and Childbirth* ©http://www.biomedcentral.com/1471-2393/3/7 (Accessed on 13/03/06 at 4:15pm)

Amorcho, B., Gomez, E., Pontes, L., Campos, I. and Landeras, J. (1999). Does the selection of catheter for embryo transfer affect the success rate of an ART unit? [Abstract] 15th Annual Meeting of the ESHRE (Tours, France). *Hum. Reprod.* Vol. **(14)**, no.**(1)**: pp. 205.

Andersen, A., Yue, Z., Meng, F., and Peterson, K. (1994). Low implantation rate after in vitro fertilization in patient with hydrosalpinx diagnosed by ultrasonography. *Hum. Reprod.* Vol. **(9)**: pp. 1935-1938.

Andersen, A.N., Lindhard, A., and Loft, A. (1996).The infertile patient with hydrosalpinges—IVF with or without salpingectomy? *Hum. Reprod.* Vol. **(11)**:pp. 2081–2084.

Anderson, R. E., Nugent, A.T., Gregg, N. L., Nunn, S. L., and Behr, B.R., and Amanda, S. L. (2002).Transvaginal ultrasound guided embryo transfer improves the outcome in patient with previous failed in vitro-fertilization cycle .*Fertil. Steril.*; Vol. **(77)**, no. **(4)**: pp.769-775.

Anguiano, A., Oates, R.D., Amos, J.A., Dean, M., Gerrard, B., and Stewart, C. (1992).Congenital bilateral absence of the vas deferens. A primarily genital form of cystic fibrosis. *JAMA.* Vol. **(267)**: pp.1794–1797.

Anselmo, A.P., Cavalier, E., and Maurizi, R.M. (1999). Hodgkin's disease during pregnancy: diagnostic and therapeutic management. *Fetal Diagn Ther.* Vol. **(14)**: pp.102-105.

Anttilla, L., Penttilä, T.A., and Suikkari, M. A. (1999). Successful pregnancy after in-vitro fertilization and transmyometrial embryo transfer in a patient with congenital atresia of cervix. *Hum.Reprod.* Vol.**(14)**, no. **(6)**: pp.1647-1649.

Asaad, M., and Carver, J.A. (1997).Twin pregnancy following transmyometrial– Sub endometrial embryo transfer for repeated implantation failure. *Hum. Reprod.* Vol.**(12)**, no. **(12)**: pp. 2824–2825.

Asch, R.N., Ellsworth, L.R., Balmaseda, J.P., and Wong, P.C. (1984). Pregnancy after translaparoscopic gamete intrafallopian transfer. *Lancet.* Vol. **(2)**: pp.134.

Ashwood–smith, M. J. (1986). *Hum. Reprod.* Vol.**(1)**: pp.319 –332. In Edwards, R. G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction. Chapter **(8)**: Oocyte growth, maturation, and fertilization: pp. 335-339.

Assisted conception program, intracytoplasmic sperm injection (ICSI) ©http://www.edinburghivf.org/icsi.html (Accessed on 24/08/06, at 3.22 pm)

(Auguste Comte 1798-1857)
©http://www.bolender.com/Sociological%20Theory/Comte,%20Auguste/comte
,_auguste.htm (Accessed on 25/09/06 at 3.45pm)

Austin, C.R. (1951). Observation of penetration of the sperm in to the mammalian egg. *Aust. J. Science. Res.* Vol. **(134)**: pp. 1581.

Awonuga, A., Nabi, A., Govindbhair, J., Brich, H., and Stewart, B. (1998).Contamination of embryo transfer catheter and treatment outcome in vitro fertilization. *Assisted Reproduction Genetics.* Vol. **(15)**: pp. 198-201.

Ayoubi, J. M., Fanchin, R., Kaddouz, D., Frydman, R., and de Ziegler, D. (2001). Uterorelaxing effects of vaginal progesterone: comparison of two methodologies for assessing uterine contraction frequency on ultrasound scans.*Fertil. Steril.* Vol.**(76)**, no. **(4)**: pp. 736-740.

Backos, M., Rai, R., and Regan, L. (2002).Antiphospholipid antibodies and infertility.*Hum. Fertil.* (Camb).Vol. **(5)**, no. **(1)**:pp.30-34

Bacon, C. R., Morrison, J. J., and O Reilly, G. (1995). ETA and ETB endothelin receptors in human myometrium characterized by the subtype selective ligand BQ 123, BQ 3020, FR 139317 and PD 151242. *J. Endocrinol.* Vol. **(144)**: pp.127-134.

Balasch, J., Fábregues, F., and Creus, M. (1992a). The usefulness of endometrial biopsy for luteal phase evaluation in infertility. *Hum. Reprod.* Vol. **(7)**: pp. 973–977.

Balasch, J., Creus, M., and Fabregues, F. (1996). Intravenous immunoglobulin preceding in vitro fertilization embryo transfer for patient with repeated failure of embryo transfer. *Fertil. Steril.* Vol. **(65)**, no. **(3)**: pp. 655-658.

Balmaceda, J.P., Bernardini, L., Ciuffard, I., Felix, C., Ord, R., and Asch, R.H. (1994).Oocyte donation in humans: a model to study the effect of age on embryo implantation rate. *Hum. Reprod.* Vol. **(9)**: pp. 2160–2163.

Barber, D., Egan, D., Rosa, C., Evan, B., and Barlow, D. (1996). Nurses performing embryo transfer: successful outcome of in vitro fertilization. *Hum. Reprod.* Vol. **(11)** : pp.105-108.

Bartosik, D. S., Jacobs, L., and Kelly, L.J. (1986). Endometrial tissue in peritoneal fluid. *Fertil. Steril.* Vol. **(46)** : pp. 796–800.

Battaglia, C., Regnani, G., Marsella, T., Facchinetti, F., Volpe, A., Ventuoli, S., and Flamigni, C. (2002). Adjuvant L-arginin treatment in controlled ovarian hyprestimulation. *Hum. Reprod.* Vol. **(17)**, no. **(3)**: pp. 659-665 http://humrep.oxfordjournals.org/cgi/reprint/17/3/659.pdf (Accessed on 11th of Feb.06)

Bavister, B.D. (1995).Culture of pre-implantation embryos: facts and artefacts. *Hum. Reprod.* ;Update. Vol. (1): pp. 91–148.

Bedford, J.M. (1990). Fertilization mechanism in man and animals: Current concept .In Edward RG (Ed) .Establishing a successful human pregnancy. *New York, Raven press.* pp. 115-132.

Bellinge, B.S., Copeland, C. M., and Thomas, T.D. (1986). The influence of patient insemination on the implantation rate in an in vitro fertilization and embryo transfer program . *Fertil. Steril.* Vol. (46): pp. 252-256.

Bennett, S., Waterston, J., and Parsons, J. (1993).Two cases of cervical pregnancy following in vitro fertilization and embryo transfer to the lower uterine cavity. *J Assist Reprod Genet.* Vol. (10): pp. 100–103.

Beral, V., and Doyle, P. (1990). Report of the MRC working party on children conceived by *in vitro* fertilisation. Births in Great Britain resulting from assisted conception, 1978–87. *British Medical Journal.* (300): pp.1229–1233.

Bergendal, A., Naffah, S., Nagy, C., Bergqvist, A., Sjöblom, P., and Hillensjö, T. (1998). Outcome of IVF in Patients with Endometriosis in Comparison with Tubal-Factor Infertility. *Journal of Assisted Reproduction and Genetics.* Vol. (15), no. (9): pp. 530 – 534.

Bergh,C., Howles,C.M., Borg,K., Hamberger, L., Josefsson B., and Nilsson, L. (1997). Recombinant human follicle stimulating hormone (r-hFSH; Gonal-F®) versus highly purified urinary FSH (Metrodin HP®): results of a randomized comparative study in women undergoing assisted reproductive techniques, *Hum. Reprod.* Vol.(12): pp. 2133–2139.

Biervliet, F.P., Lesny, P., Maguiness, S.D., Robinson, J., and Killick, S.R. (2002). Transmyometrial embryo transfer and junctional zone contractions *Hum. Reprod.*Vol. 17, no. (2): pp. 347-350.

Bischoft and Barry (1840). In Fishel, S. and Symonds, E.M. (1986).In vitro fertilization past present and future; IVF historical perspective: pp. 3

Blake, D., Proctor, M., Johnson, N., Olive, D. (2005). Cleavage stage versus blastocyst stage embryo transfer in assisted conception. Cochrane Database Syst Rev. Oct 19 ;( 4):CD002118.

Blazer, A.S., Hogan, J.W., and Seifer, D.B. (1997).The impact of hydrosalpinx on successful pregnancy in tubal factor infertility treated by in-vitro fertilization. *Fertil. Steril.* Vol. (67): pp. 517–520.

Bleil, J.D., and Wassermann, P.M. (1986). Auto radiographic Visualization of the Mouse Egg's Sperm Receptor Bound to Sperm .*Journal of Cell Biology.* Vol. (**102**): pp.1363-1371.

Bloechle, M., Schreiner, T., and Lisse, K. (1997). Recurrence of hydrosalpinges after transvaginal aspiration of tubal fluid in an IVF cycle with development of a serotometra. *Hum. Reprod.* Vol. (**12**): pp.703–705.

Botta, G., and Grudzinskas, G. (1997). Is a prolonged bed rest following embryo transfer useful? *Hum. Reprod.* Vol. (**12**) : pp. 2489-2492.

Braeckmans, P., Devroey, P., Camus, M., Khan, I., Staessan, C., Smitz, J., Van Waesberghe, L., Wisanto, A., and Van Steirteghem, A. C. (1987). Gamete intra-Fallopian transfer: evaluation of 100 consecutive attempts. *Hum. Reprod.* Vol. (**2**), no. (**3**): pp. 201-205.

Brinholz, J. (1984). Ultrasound visualization of the endometrial movement. . *Fertil. Steril.*; Vol. (**41**): pp. 157-158.

Brinsden, P. (1999).An introduction to Bourn Hall. In Text book of In Vitro Fertilization and assisted reproduction, chapter (**1**): pp. 5.

Broekmans, F.J. and, Klinkert, E.R.(2004) Female Age in ART: When to Stop? *Gynecologic and Obstetric Investigation.* Vol. (**58**), no. (**4**):pp.225-234

Brosens, J.J., De Sousa, N.M., and Barker, F.G. (1995).Uterine junctional zone: function and disease. *Lancet.* Vol. (**346**): pp.558 -560

Brosens, J.J., Barker, G., and de Sousa, M. (1998). Myometrial zonal differentiation and uterine junctional zone hyperplasia in the non pregnant uterus. *Hum. Reprod. Update*, Vol.(**4**), no. (**5**): pp. 496-502.

Brossfield, J.E., Chan, P.J., Patton, W.C., and King, A. (1999). Tenacity of exogenous human papillomavirus DNA in sperm washing. *J. Assist. Reprod. Genet.* Vol. (**16**): pp. 325–328.

Brown, H.K., Stoll, B.S., and Nicosia, S.V. (1991).Uterine junctional zone: correlation between histological findings and MRI. *Radiology*; Vol. (**179**): pp. 409–413.

Bulletti, C., Rossi, S., and de Ziegler, D. (1997).The uterine contractility in endometriosis. [Abstr. ] In *International Meeting on Infertility and Assisted Reproductive Technology, Porto Cervo, Italy, June 11–14, 1997, Abstract Book* :pp. 129.

Bungee, R.G., and Sherman, J.K. (1953). Fertilizing capacity of frozen spermatozoa. *Nature.* Vol. (**172**): pp. 767.

Cahill, D.J., Prosser, C.J., Wardle, P.G., Ford, W.C.L., and Hull, M.G.R. (1994). Relative influence of serum follicle stimulating hormone, age, and other factors on ovarian response to gonadotrophin stimulation. *BrJ Obstet Gynaecol*; Vol. **(101)**: pp. 999–1002.

Carrillo, A.J., Pridham, D., Risch, P., Pool, T., Silverman, I., and Christine, L. (1998). Improved clinical outcome for invitro fertilization with delay of embryo transfer from 48 to 72 hrs: after oocyte retrieval use of glucose and phosphate free media. *Fertil. Steril.*Vol.**(69)**, no. **(2)**: pp. 329-343.

Chang, M.C. (1951). Fertilization capacity of spermatozoa deposited in the fallopian tube. *Nature.*Vol. **(168)**: pp. 697.

Chang, M. C. (1959). Fertilization of rabbit ova in vitro *.Nature* (London); Vol. **(184)**: pp. 406.

Charbonnel, B., Krempf, M., Blanchard, P., Dano, F., and Delage, C. (1987). Induction of ovulation in polycystic ovary syndrome with a combination of a Lutinising hormone – releasing hormone analogue and exogenous gonadotrophin. *Fertil. Steril.* Vol. **(47)**: pp. 920-924.

Check, J.H., Nowroozi, K., Chase, J., Nazari, A., and Braithwaite, C. (1992). Comparison of pregnancy rates following in vitro fertilization-embryo transfer between the donors and the recipients in a donor oocyte program. *J. Assist. Reprod. Genet.* Vol. **(9)**, no. **(3)**: pp. 248-250.

Check, J.H., O'Shaughnessy, A., Lurie, D., Fisher, C., and Adelson, H.G. (1995). Evaluation of the mechanism for higher pregnancy rates in donor oocyte recipients by comparison of fresh with frozen embryo transfer pregnancy rates in a shared oocyte programme. *Hum Reprod.* ; Vol.**(10)**, no. **(11)**: pp. 3022-3027.

Check, J.H., Choe, J.K., Katsoff, D., Summers-Chase, D., and Wilson, C. (1999). Controlled ovarian hyprestimulation adversely affects implantation    following in vitro fertilization-embryo transfer. *J. Assist. Reprod. Genet.* Vol. **(16)**: pp. 416-420.

Check, J.H., Choe, J.K., Nazari, A., Fox, F., and Swenson, K.(2001).Fresh embryo transfer is more effective than frozen for donor oocyte recipients but not for donors. *Hum. Reprod.*Vol. **(16)**, no. **(7)**:pp.1403-1408.

Chen, C. (1986). Pregnancy after human oocyte cryopreservation, *Lencet,* **(1)**:pp. 884.From    History-    20th    Century.    UK.    Available    from: www.repromed.org.uk/history/20th_uk.htm .(Accessed on 21/08/06 at 1.30 pm)

Chien, L., Kien, H., Xiao, J., and Tzeng, CR. (2002). Fluid accumulation within the uterine cavity reduces pregnancy rate in women undergoing IVF. *Hum. Reprod.* Vol. **(17)**, no. **(2)**:pp. 351-356.

Choe, J. K., Nazari, A., Check, J. H., Summers-Chase D., and Swenson, K. (2001). Marked improvement in clinical pregnancy rates following in vitro fertilization–embryo transfer seen when transfer technique and catheter were changed, *Clin. Exper. Obstet. Gynecol.* **(28):** pp. 223–224.

Clapp, D. (1985). Emotional responses to infertility. Nursing interventions. *J.Obsiet. GynecoL Neonatal Nurs.* Vol. **(14),** no. **(6):**pp. 32-35.

Cohen, J., Simons, R.F., and Edwards, R.G. (1985). Pregnancies following the frozen storage of expanding human blastocysts. *J. In Vitro Fertil. Embryo Transfer.* Vol. **(2):** pp. 59–64.

Cohen, J., Elsner, C., Korl, H., Malter, H., Massey, J., Mayer, M.P., and Weimer, K. (1990). Improvement of implantation by assisted hatching using micro manipulation. *Hum. Reprod.* Vol. **(5):** pp. 7-13.

Cohen, J., Elsner, C., Kort, H. (1990).*Hum.Reprod.;*(**5**): pp.7-13. In Edwards, R.G., Brody, S.A. (1995).Principles and practice of assisted human reproduction. Chapter **11:** Implantation rates during IVF, GIFT, and other forms of assisted conception: pp. 502.

Cohen, J., Adler, A., Alikano, M., Ferrara, T.A., Kissin, E., Reing, A.M., Suzman, M., Talansky, B.E., Rosenwaks, Z. (1993).Assisted fertilization and abnormal sperm function. Semen *Reprod Endocrino.* Vol. **(11):**pp. 83-94.

Cohen, J., Gilligan, A., Esposito, W., Schimmel, T., and Dale, B. (1997). Ambient air and its potential effects on conception in vitro. *Hum. Reprod.* Vol. **(12):**pp. 1742–1749.

Cohen, J. (1998). Embryo replacement technology. San Francisco 31 Annual post graduate course. ASRM . In Mansour, R.T., Aboulghar, M.A. (2002); Optimizing the embryo transfer technique. *Hum. Reprod.* Vol. **(17),** no. **(5):**pp.1149-1152.

Cohen, J., Trounson, A., Dawson, K., Jones, H., Hazekamp, J., Nygren, K.G., and Hamberger, L.(2005).The early days of IVF outside UK. *Hum. Reprod. Update* Vol.**(11),**no.**(5):**pp.439-59.

Cornilli, F.J., Lauwweryns, J.M., and Brosens, I.A. (1985).*Gynaecol. Obstet. Invest.*; **20:** 113-129. In Edward R.G., and Brody S.A.(1995). Principles and practice of assisted human reproduction; Chapter **(11):** Implantation rates during IVF, GIFT, and other form of assisted conception: pp. 495.

Coroleu, B., Carreras, O., and Veiga, A. (2000). Embryo transfer under ultrasound guidance improve pregnancy rate after in-vitro fertilization .*Hum.Reprod.* Vol. **(15):** pp. 616-620.

Coroleu, B., Barrie, N. P., Carreras, O., Martinez, F., Parriego, M., Hereter, L., Parera, N., Veiga, A., and Balasch, J. (2002). The influence of the depth of embryo replacement in to the uterine cavity on the implantation rate after IVF a controlled ultrasound guided study. *Hum. Reprod.* Vol.(17), no. (2): pp. 341-346.

Craft, I., McLeod, F., and Edmonds, K. (1981) Human embryo transfer technique. *Lancet.* Vol.(2): pp. 1104–1105.

Cross, N.L., and Hanks, S.E. (1991); *Human Reprod.* (6):1279-1283. In Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction. Chapter 3: Spermatogenesis, ejaculation, and spermatozoa: pp. 93.

Csapo, A.I. (1980). A rationale for the treatment of dysmenorrhoea. *J Reprod Med*; Vol.25, no. (4):pp. 213–221.

Csemiczky, G., Landgren, B.M., and Fried, G. (1996). High tubal damage grade associated with low pregnancy rate in women undergoing in-vitro fertilization treatment. *Hum. Reprod.* Vol. (11): pp. 2438–2440.

Cummins, J.M., Breen, T.M., Harrison K.L., Shaw, J.M., Wilson, L.M., and Hennessey J.F. (1986). A formula for scoring human embryo growth rates in in vitro fertilization: its value in predicting pregnancy and in comparison with visual estimates of embryo quality. *Journal of In Vitro Fertility and Embryonic Transfer.* Vol. (3): pp.284–295.

Dawson, K.J., Conaghan, J., Ostera, G.R., Winston, R.M., and Hardy, K. 1. (1995). Delaying transfer to the third day post insemination, to select non-arrested embryos, increases development to the foetal heart stage. *Hum. Reprod.* Vol. (10): pp.177–182.

De La Rochebrochard, E., de Mouzon, J., Thepot F., Thonneau, P. (2006); French National IVF Registry (FIVNAT) Association.Fathers over 40 and increased failure to conceive the lessons of invitro fertilization in France. *Fertil. Steril.*Vol.(85),no.(5):pp.1420-1424.

Demas, B.E., Hricak, H., and Jaffe, R.B. (1985). Uterine MR imaging: effects of hormonal stimulation .*Radiology.* Vol (159): pp. 123-126.

De Mouzon, J., Thepot F., Thonneau, P. (2006); French National IVF Registry (FIVNAT) Association.Fathers over 40 and increased failure to conceive The lessons of invitro fertilization in France. *Fertil Steril.*Vol.(85),no.(5):pp.1420-1424.

Derom, C., Vlietinck, R., Derom, R., Van den Berghe, H., and Thiery, M. (1987). Increased monozygotic twinning rate after ovulation induction. *Lancet.* Vol. (1): pp.1236-1238.

Devreker, F., Emiliani, S., and Revelard, P. (1999). Comparison of two elective transfer policies of two embryos to reduce multiple pregnancies without impairing pregnancy rates. *Hum. Reprod.*Vol. (14): pp. 83–89.

De Vries, K., Lyons, E.A., Ballard, G., Levi, C.S., and Lindsay, D.J. (1990).Contractions of the inner third of the myometrium. *Am. J. Obstet. Gynecol.* Vol. (162): pp. 679- 682.

Devroey, P., Braeckmans, P., Smitz, J., Van Steirteghem, A., Heytens L., and Camu, F. (1986). Pregnancy after Tran's laparoscopic zygote intra fallopian transfer in patient with sperm antibodies. *Lancet.* Vol. (1): pp. 1329.

Devroey, P., Camus, M., Staessen, C., Wisanto, A., Bollen, N., Henderix, P., and Van Steirteghem, A.C. (1990); ZIFT: indication and limitation. In Capitanio GL, Asch RH, De Cecco L, Croce S. (Eds) GIFT: from Basic to Clinics, *New York: Raven Press*: pp. 333-340.

Diedrich, K., Van der Ven, H., Al Hasani, H., and Krebs, D. (1989). Establishment of pregnancy related to embryo transfer technique after in vitro fertilization. *Hum. Reprod.* Vol. (4): pp. 111-114.

Dockery, P., Warren, M.A., and Li, T.C. (1990). *Hum .Reprod.* Vol. (5): pp. 494-498. In Edwards, R.G., and Brody, S.A.,(1995).Principles and practice of assisted human reproduction. Chapter (11): Implantation rates during IVF, GIFT, and other forms of assisted conception: pp. 495-500.

Dorn, C., Reins berg, J., Herald, S., Prietl, G., Vander Van, H., and Krebs, D. (1999). Serum Oxytocin concentration during embryo transfer. *European Journal of Obstetric and Gynaecology and Reproductive Biology.* Vol. (87): pp.77-80.

Downie, J., Poyser, N.L., and Wanderlich, M. (1974); Level of prostaglandin in human endometrium during the normal menstrual cycle *.J. Physiology.* Vol. (236): pp. 465.

Drevius, L.A. (1972).The sperm raise test. *J. Reprod. Fertil.* Vol. (24): pp. 427- 432.

Dunson, D.B., Colombo, B., and Baird, D.D. (2002).Changes with age in the level and duration of fertility in the menstrual cycle. *Hum. Reprod.* Vol. (17):pp.1399–1403

Ebner, T., Yaman, C., Moser, M., Somme, Gruber, M., Pölz, W., and Tews, G. (2001).The ineffective loading process of the embryo transfer catheter alters implantation and pregnancy rates. *Fertil. Steril.* Vol. (76), no. (3): pp. 630-632.

Edirisinge, W., Junk, S.M., and Matson, P.L. (1997). Birth from cryopreserved embryos following in-vitro maturation of oocytes and intracytoplasmic sperm injection. *Hum. Reprod.* Vol. (12): pp. 1056–1058.

Edwards, R.G. (1956). *Nature*; Vol. **(208)**: 349-351. In Edwards, R.G., and Brody, S.A. (1995).Principles and practice of assisted human reproduction; Chapter **(2)**: History and ethics of assisted human conception: pp. 17-45.

Edwards, R.G., and Gate, A.H. (1959). *J.Endocrinol*;Vol.**(18)**:292-304. In Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction. Chapter **(2)**: History and ethics of assisted human conception: pp. 17- 45.

Edwards, R.G. (1965). Maturation in vitro of human oocyte .*Lancet*; Vol. **(2)**: pp. 926-929. In Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction. Chapter **(2)**: History and ethics of assisted human conception: pp.17-45.

Edwards, R.G., Bavister, B.D., and Steptoe, P.C. (1969). *Nature* **221**:632-635.In Edwards, R.G., and Brody, S.A. (1995).Principles and practice of assisted human reproduction. Chapter **(2)**: History and ethics of assisted human conception: pp.22.

Edwards, R.G., Steptoe, P.C., and Purdy, J.M. (1980).Establishing full-term human pregnancies using cleaving embryos grown in vitro. *Br J Obstet Gynaecol*. Vol. **(87)**: pp. 737–756.

Edwards, R.G., Donahue, R.P., Baramuki, T.A., and Jones, H.W. (1981). Preliminary attempt to fertilize human oocyte matured in vitro. *Am. J. Obst. Gynae*. Vol. **(1)**: pp. 1163.

Edwards, R.G., Fishel, S.B., and Cohen, J. (1984). *J. In vitro Fertil, embryo Transfer*. Vol. **(1)**: pp.3-23. In Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction; Chapter **(2)**: History and ethics of assisted human conception: pp.26.

Edwards, R.G., Mettler, L., and Walters, D. E. (1986). Identical twins and in vitro fertilization. *Journal of In Vitro Fertility and Embryonic Transfer;* Vol. **(3)**: pp.114–117.

Edwards, R.G., and Bordy, S.A. (1995). Principles and practice of assisted human reproduction, chapter **(2)**: History and ethics of assisted human conception: pp. 17-45.

Edwards, R.G., and Bordy, S.A. (1995). Principles and practice of assisted human reproduction, chapter **(3)**: Spermatogenesis, ejaculation, and spermatozoa: pp.49-102.

Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction, chapter **(8)**: Oocyte growth, maturation and fertilization: pp. 336-344.

Edwards, R.G., and Bordy, S.A. (1995). Principles and practice of assisted human reproduction, chapter **(9)**: Human fertilization in the laboratory : pp. 351- 407.

Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction. Chapter **(10)**: The human embryo in vivo and in vitro: pp. 415-468.

Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction, chapter **(11)**: Implantation rates during IVF, GIFT, and other forms of assisted conception

Edwards, R. G., Lobo, R., and Bouchard, P. (1996). Time to revolutionize ovarian stimulation, *Hum. Reprod.* Vol. **(11)**: pp. 917– 919.

Egan, D.M., Gosden, B., King BridgeWood, E.A., Robinson, J., Ross, C.F., and Barlow, D.H. (1990). Difficult embryo transfer influence on in vitro fertilization. Abstracts of the Joint ESHRE Meeting, Milan *Hum Reprod.* Vol **(5)**: pp. 84.

Egbase, P.E., al Sharhan, M., al Othman, S., al Mutawa, M., Udo, E.E., and Grudzinskas, J.G. (1996). Incidence of microbial growth from the tip of the embryo transfer catheter after embryo transfer in relation to clinical pregnancy rate following in-vitro fertilization and embryo transfer. *Hum. Reprod.* Vol. **(11)**: pp.1687–1689.

Egbase, P.E., al Sharhan, M., Udo, E.E., and Grudzinskas, J.G. (1999). Prophylactic antibiotics and endocervical microbial inoculation of the endometrium at embryo transfer. *Lancet.* Vol. **(354)**: pp. 651–652.

Einspanier, A., Bielefeld, A., and Kopp, H. (1998); Expression of the oxytocin receptor in relation to steroid receptor in the uterus of the primate model, the marmoset monkey. *Hum. Reprod.* Update, Vol.**(4)**, no. **(5)**: pp. 634-646.

Embrey, M. (1971). PGE compound for induction of labour and abortion. *Academic science.* Vol. **(180)**: pp. 518-523.

Embryo culture and transfer (Day3 or 5) Victoria fertility centre ©http://www.victoriafertility.com/services/embryo-culture-transfer.htm(Accessed on 29/08/06 at 11:32am)

Englert, Y., Puissant, F., Camus, M., Van Hoeck, J., and Leroy, F. (1986). Clinical study on embryo transfer after human in vitro fertilization. *J. In Vitro Fertil. Embryo Transfer.* Vol. **(3)**: pp. 243-246.

Espy, L.L. (1992). Ovulation as an inflammatory process. In Sjoberg N.O. Hamberger, L, Janson , P.O. et al (eds), Local regulation of ovarian function , Park Ridge, NJ: Parthenon Publishing Group, UK : pp.183–201.

European IVF-monitoring program European Society of Human Reproduction and Embryology Nygren KG, Andersen AN, EIM Program (2002). Assisted reproductive technology in Europe 1999. Results generated from European registers by ESHRE. *Hum Reprod.*Vol. (17): pp. 3260–3274.

Evans, J., Wells, C., and Hood, K. (1999).A possible effect of different light sources on pregnancy rates following gamete intra-Fallopian transfer. *Hum Reprod.*Vol. (14): pp. 80–82.

Fanchin, R., Righini, C., O livennes, F., and Frydman, R. (1997). Uterine contractions as visualised by ultrasound alter pregnancy rates in IVF and embryo transfer. *Hum. Reprod.* Vol. (12): pp. 33–34.

Fanchin , R., Harmas , A., and Benaoudia , F. (1998a). Microbial flora of the cervix assessed at the time of embryo transfer adversely affects in vitro fertilization outcome. *Fertil. Steril.* Vol. (70): pp. 866- 870.

Fanchin, R., Righini. C., Olivennes F., Taylor, S., and de Ziegler, D. (1998b). Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization.*Hum.Reprod.* Vol. (13), no. (7): pp. 1968 -1974.

Fanchin, R., Ayoubi, J.M., Righini, C., Olivennes, F., Schonauer, L.M., and Frydman, R. (2001a).Uterine contractility decreases at the time of blastocyst transfer. *Hum.Reprod.*Vol.(16), no. (6): pp. 1115-1119.

Fanchin, R., Righini, C., Ziegler, D., Olivennes, F., Leede, N., and Frydman, R. (2001b). Effect of vaginal progesterone administration on uterine contractility at the time of embryo transfer. *Fertil. And Steril.* Vol (75), no. (6): pp. 1136-1140.

Farhi, J., Weissman, A., Nahum, H., and Levran, D. (2000). Zygote intra fallopian transfer in patient with tubal factor infertility after repeated failure of implantation with invitro fertilization embryo transfer. *Fertil. Steril.*Vol. (74), no. (2): pp. 390-393.

Fehilly, C.B., Cohen, J., and Simons, R.F. (1985).Cryopreservation of cleaving embryos and expanded blastocysts in the human: a comparative study. *Fertil. Steril.*Vol. (44): pp. 638–644.

Feichtinger, W. and Kemeter, P. (1984). Laparoscopic or ultrasonically guided follicle aspiration for in vitro fertilization*? J. Of assisted reproduction and genetics.* Vol.(1),no.(4):pp.244-249.

Feichtinger, W., Barad, D., Feinman, M., and Barg, P. (1990). The use of two-component fibrin sealant for embryo transfer. *Fertil. Steril.* Vol.(54): pp.733-734.

Feichtinger, W., Strohmer, H., Radner, K.M., and Goldin, M. (1992).The use of fibrin sealant for embryo transfer: development and clinical studies. *Hum. Reprod.* Vol.(7): pp.890–893.

Filicori, M., Cognigni, G.E., and Taraburelli, S. (1999). Lutinising hormone activity supplementation enhance follicular stimulating hormone efficacy and improve ovulation outcome .*J. Clinical Endocrinology metab*. Vol.(84) : pp. 2659-2663.

Fishel, S., and Edwards, R.G. (1983). In vitro fertilization of human oocytes: Factors associated with embryonic development in vitro, replacement of embryos and pregnancy. In Beier, H.M. and Lindner, H.R. (Eds), *Fertilization of the Human Egg in vitro*. Springer-Verlag, Berlin, Germany: pp. 251–269.

Fishel, S., and Symonds, E. (1986). In vitro fertilization past present future, historical perspective: pp. 1-16.

Fishel, S., Webster, J. Jackson, P., and Faratian, B. (1989). Evaluation of high vaginal insemination at oocyte recovery in patients undergoing in vitro fertilization. *Fertil. Steril*. Vol. (51): pp. 135-138.

Fishel, S.B., Webster, J., Faratian, B., Jackson, P., Shelton, K.. and Johnson, J.(1991). Establishing pregnancies after follicular stimulation for IVF with clomiphene citrate and human menopausal gonadotrophin only. *Hum. Reprod.* Vol. (6): pp.106-112.

FIVNAT (French In vitro National). (1995). Pregnancies and births resulting from in vitro fertilization. *Fertil. Steril.* Vol.(64): pp.746–756.

Fleming, R., Adam, A.H., Barlow, D.H., Black, W.P., MacNaughton, M.C., and Coutts, J.R.T. (1994). Anew systematic treatment for infertile women with abnormal hormone profile *Br. J. Obstet. Gynaecol.* Vol. (89): pp. 80 – 83.

Florman, H. M., and Wassermann, P. M. (1985) O-Linked oligosaccharides of mouse egg ZP3 account for its sperm receptor activity. *Cell.* Vol. (41):pp. 313-324.

Fox, C. A., Wolff, H. S., and Baker, J. A. (1970). Measurement of intra - vaginal and intra- uterine pressures during human coitus by radio – telemetry. *J. Reprod. Fertil.* Vol. (22): pp. 243-251.

Franco, G., Martinez, C., Mauri, L., Peterson, G., Felipe, V., Contart, P., Pontes, A., and Oliveira, A. (2004).Best site for embryo transfer the upper or the lower half of endometrial cavity. *Hum. Reprod.*Vol.(19), no. (8): pp. 1785-1790.

Fraser, L.R., and Ahuja, K.K. (1988) *Gamete Res.*; (20): pp.491. In Edwards R.G., and Brody, S.A.(1995). Principles and practice of assisted human reproduction, chapter (8): Oocyte growths, maturation and fertilization: pp. 309.

Fraser, I.S. (1992). Prostaglandin inhibitors and their roles in gynaecological disorders. *Baillieres Clin. Obstet. Gynecol.*Vol. (6): pp. 829–857.

Freeman, M.R., Whitworth, C.M., and Hill, G.A. (1998); Permanent impairment of embryo development by hydrosalpinges. *Hum. Reprod.* Vol.(**13**), no. (**4**):pp. 983-986.

Friedler, S., Giudice, L., and Lamb, E. (1988).Cryopreservation of embryos and ova. *Fertil. Steril.* Vol. (**49**): pp. 743–764.

Fukuda, M., and Fukuda, K. (1994).Uterine endometrial cavity and cervical mucus . *Hum. Reprod.* Vol.(**9**), no. (**6**): pp. 1013-1016.

Fujiwara, T., Togashi, K., Yamaoka, T., Nakai, A., Kido, A., Nishio, S., Yamamoto, T., Kitagaki H., and Fujii S. (2003). Kinematics of the Uterus: Cine Mode MR Imaging *Radiographic.* Vol. (**24**): pp. 19.

Gallardo, E., Simon, C., Levy, M., Guanes, P.P., Remohi, J., and Pellicer, A.(1996). Effect of age on sperm fertility potential: oocyte donation as a model. *Fertil. Steril.* Vol. (**66**), no. (**2**): pp. 260-264.

Gardner, R. M., and Stancel, G. M. (1989). Blockade of epidermal growth factor – induced uterine contractions by indomethacin and nordihydroguaritic *acid J. Pharmacol. Exp.Ther.* Vol. (**258**): pp. 882-886.

Gardner, D.K., and Lane. M. (1999). Embryo culture systems. In: Trounson, A.O., Gardner, D.K., eds. Handbook of In Vitro Fertilization, Second Edition. Boca Raton: CRC Press: pp. 205-264.

Gardner, D.K., and Schoolcraft, W.B. (1999). In vitro culture of human blastocyst. In: Jansen, R., Mortimer, D., (Eds) towards reproductive certainty: infertility and genetics beyond. Carnforth, Parthenon Press, 1999: pp. 378–388.

Gardner, D.K. (2000) Blastocyst culture: Toward single embryo transfers. *Hum. Fertil.* Vol.(**3**), no. (**4**): pp. 228-237.

Gardner, D.K., Phil, D., Surrey, E., Minjarez, D., Leitz, A., Stevens, J., and Schoolcraft, W. (2004). Single blastocyst transfer: a prospective randomized trial. *Fertil. Steril.*Vol. (**81**): pp. 551–555.

Geimonen, E., Jiang, W., and Ali, M. (1996). Activation of protein – kinase –C in human uterine smooth muscle induces connexion -43 gene –transcription through an AP-1 site in the promoter sequence .*J. Biol. Chemi.* Vol. (**271**): pp. 23667-23674.

Gemzell, C.A., Diczfalusy, E., and Tillinger, K.G. (1958). Clinical effect of human pituitary follicle-stimulating hormone, *J Clin Endrcrinol Metab.* Vol. (**18**): pp. 1333.

Genetics and IVF institutes : Life begin at Genetics and IVF Institute (BLASTOCYST STAGE EMBRYO TRANSFER) ©http://www.givf.com/blastocyst.cfm (Accessed on 01/06/06 at 9:15am)

Gerris, J., De Neubourg, D., Mangelschots, K., Van Royen, E., Van de Meerssche, M., and Valkenburg, M. (1999). Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. *Hum. Reprod.* Vol. **14**, no. **(10)**: pp. 2581-2587.

Gerris, J. M. (2004). Single embryo transfer and IVF/ICSI outcome: a balanced appraisal. *Hum. Reprod.* Vol.**11**, no. **(2)**: pp. 105-121.

Gerris, J., and Neubourg, D. (2005). Single embryo transfer after IVF/ICSI: Present possibilities and limits. *Obstet. Gynecol. India.* Vol. **(55)**, no. **(1)**: pp. 26-47.

Gestaldi, C., Loda, G., and Salvi, R. (1993). Preliminary report on intra endometrial embryo transfer (IET). *Hum. Reprod.*Vol. **(8)**, no. **(1)**: pp. 37.

Ghazzawi, I.M., Al Hasani, K.R., and Souso, S. (1999). Transfer technique and catheter choice influence the incidence of Trans cervical embryo expulsion and the outcome of IVF .*Hum. Reprod.*Vol. **(14)**: pp. 677- 682.

Gibson, F.L., Ungerer, J.A., Leslie, G.I., Saunders, D.M., and Tennant, C.C. (1998). Development, behaviour and temperament: a prospective study of infants conceived through in-vitro fertilization. *Hum. Reprod.* Vol. **(13)**: pp.1727–1732.

Gleicher, N., Friberg, J., and Fullan, N. (1983). Egg retrieval for in vitro fertilisation sonographically controlled vaginal culdocentesis, *Lancet.* Vol. **(2)**: pp. 508.

Gmachl, M., and Kreil, G. (1993).Bee venom hyaloronidase is homologous to a membrane protein of mammalian sperm. Proc. *Natl. Acad. Sci.* Vol. **(90)**: pp.3569-3573.

Gonen, Y., Dirnfeld, M., and Goldman, S. (1991). Does the choice of catheter for embryo transfer influence the success rate of in-vitro fertilization? *Hum.Reprod.* Vol. **(6)**: pp. 1092-1094.

Goudas, V.T., Hammit, D., Damario, M., Session, D., Singh, A., and Dumesic, D. (1998). Blood on the embryo transfer catheter is associated with decreased rates of embryo implantation and clinical pregnancy with the use of in vitro fertilization embryo transfer. *Fertil. Steril.* Vol. **(15)**: pp. 198-201.

Grizard, G., Lombard –Vignon, N., and Boucher, D. (1992).*Hum. Reprod.* Vol. **(7)**: pp.1245-1248. In Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction. Chapter **(3)**: Spermatogenesis, ejaculation and Spermatozoa: pp. 93.

Groutz, A., Lessing, J.B., Wolfy, Y., Yovel, I., Azem, F., and Amit, A. (1997a).Cervical dilatation during ovum pick-up in patients with stenosis effect on pregnancy outcome in an in vitro fertilization embryo transfer program .*Fertil. Steril.* Vol. **(67)**, no. **(5)**: pp. 909-911.

Groutz, A., Lessing, J.B., Wolf, Y., Azem, F., Yovel, I., and Amit, A. (1997b). Comparison of transmyometrial and transcervical embryo transfer in patients with previously failed *in vitro* fertilization–embryo transfer cycles and/or cervical stenosis. *Fertil. Steril.* Vol. **(67)**: pp.1073–1076.

Grudzinskas, J.G., and Fay, T.N. (1990).Secretary proteins of the human endometrium .In Edward RG (Ed) Establishing a successful human pregnancy .*New York, Raven Press*: pp. 199.

Grunert, G.M., Dunn, R.C., Valdes, C.T., Wun, C.C., and Wun, W.S.A. (1998). Comparison of Wallace, Frydman DT and Cook embryo transfer catheter for IVF: a prospective randomised study [abstract]. *Annual Meeting of the American Society of Reproductive Medicine, 1998. Fertil. Steril.*Vol. **(70)**: pp. 120.

Gubbini, G., Tabanelli, C., Orlandi, C., Bazzochi, G., and Lanfrandi-Assuero, G. (1991).A method for evaluating human tubal motility in vivo, a new approach for infertility investigation .*Euro. Fertil.* Vol. **(22)**: pp. 315 – 319.

Guidice, L.C., and Ferenczy, A. (1996). the endometrial cycle. In Adashi, E.Y., Rock J.A., and Rosenwaks (Eds), Reproductive Endocrinology, Surgery and Technology. Lippincot *Raven, Philadelphia, a*: pp. 271-300.

Hamberger, L.; In Edward, R.G., and Purdy, J.M. (Eds)(1982). Human Conception in vitro. *London, Academic* Press: pp. 130. In Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction. Chapter **(7)**: Natural cycle and ovarian stimulation in assisted conception: pp. 233-279.

Hamish, W.W., and Thomas, W.K. (2004). Ovarian reserve and reproductive age may be determined from measurement of ovarian volume by transvaginal Sonography.*Hum. Reprod.* Vol.**19**, no. **(17)**: pp. 1612-1617.

Hammond, J. (1949): Culture of mouse embryos using an egg-saline medium, *Nature*, **163**: pp. 28: In History - 20th Century – UK, G:\History\history of IVF.htm – ©www.repromed.org.uk/history/20th accessed on 22/08/ 2006) at 6.22 pm).

Hans, G.I., Weering, V., Schats, R., McDonnell, J., Vink, J., Vermeiden, M., Jan, P.W., and Hompes, G.A. (2002). The impact of the embryo transfer catheter on the pregnancy rate in IVF. *Hum. Reprod.* Vol. **(17)**, no. **(3)**: pp. 666-670.

Hardy, K., Handyside, A.H., and Winston, R.M.L. (1989). *Development* **(107)**:pp.597-604. In Edwards, R.G., and Brody, S.A. (1995). Principles and practice of

assisted human reproduction. Chapter **(10)**.The human embryo in vivo and in vitro: pp. 417- 468.

Harper, M.J. (1994).Gamete and zygote transport in Knobil, E. Neill, J.D. (Eds). The physiology of reproduction, 2$^{nd}$, *Raven Pres. New York*: pp. 123-187.

Hassan,M.A., Killick, S.R.(2003).Effect of male age on fertility evidence for the decline in male fertility with increasing age. *Fertil.Steril.*Vol.**(79)**, no.**(3)**:pp.1520-1527

Hazout, A., and Me´ne´zo, Y. (1996). Embryonal implantation. Fundamental and practical aspects. *J. Gynecol. Obstet. Biol. Reprod.* Vol. **(25)**: pp. 862–865.

Heap, W. (1891). Preliminary note on the transplantation and growth of mammalian ova with in the uterine foster mother. *Proc. R. Soc.*; Vol. **(48)**: pp. 457-458.

Hearns-Stokes, R.M., Miller, B.T., Scoutt, L., Creuss, D., Chakraborty, P.K., and Segars, J.H. (2000). Pregnancy rates after embryo transfer depend on the provider at embryo transfer. *Fertil. Steril.*Vol. **(74)**: pp. 80–86.

Henne, M. B., and Milki, A. (2004). Uterine position at real embryo transfer compared with Mock embryo transfer. *Hum. Reprod.* Vol **(19)**, no. **(3)**: pp. 570-572.

Henry, J.S., and Browne, J.S.(1943).The contractions of the human uterus during the menstrual cycle. *Am .J. Obstet. Gynaecol.* Vol. **(45)**: pp. 927.

HFEA, 2004 ©http://212.49.193.187/cps/rde/xchg/hfea/hs.xsl/891.html(Accessed on 05/06/06 at 4.12pm)

HFEA and GMC 2004
©http://www.gmcuk.org/about/partners/human_fertilisation_and_embryology_authority.asp (Accessed on 24/08/06 at 12:12pm)

History- 20$^{th}$ Century. UK. Available from:©www.repromed.org.uk/history/20th_uk.htm [Accessed on 22/08/06 at 8.35 pm].

Hoffman, p., Stank -Labesque, F., Fanchin, R., Dilai, N., Pons, J.C., and Ayoubi, J.M. (2003). Effect of L-arginin and Sodium Nitoprusside on the spontaneous contractility of human non-pregnant uterus. *Hum. Reprod.*Vol.**(18)**, no. **(1)**: pp. 148-151.

Howlett, S.K., and Bolton, V.N. J. (1985).*Embryol. Morphol.*; Vol. **(87)**: pp.175-206: In Edwards, R. G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction. Chapter **(10)**: The human embryo in vivo and in vitro: pp. 417- 468.

Hull, M. G., Williams, J.A., Ray, B., McLaughlin, E.A., Akande, V.A., and Ford, W.C.(1998).The contribution of subtle oocyte or sperm dysfunction affecting

fertilization in endometriosis-associated or unexplained infertility: a controlled comparison with tubal infertility and use of donor spermatozoa. *Hum. Reprod.,* Vol. **(13):** pp. 1825-1830.

Human fertilization and Embryology Act 1990.London: Her Majesty stationary office, 1990.

Human Fertilisation and Embryology Authority (1996). *Fifth Annual Report.* Human Fertilisation and Embryology Authority, London.

Hurley, V.A., Osborn, J. C., and Leonie, M. A. (1991). Ultrasound –guided embryo transfer: a controlled trial .*Fertil. Steril.* Vol. **(55):** pp. 559-562.

Ijiland, M.M., Evers, J. L., and Dunselman, G. A. (1996).Endometrial wavelike movements during the menstrual cycle .*Fertil. Steril.* Vol. **(65):** pp.746-749.

Ijiland, M.M., Evers, J. L., and Dunselman, G. A. (1997a). Relation between endometrial wavelike activity and fecundability in spontaneous cycles .*Fertil .Steril.*Vol. **(67):** pp. 492-495.

Ijiland , M. M ., Evers, J. L., and  Hoagland, H. J. (1997b). Velocity of endometrial wavelike activity in spontaneous cycle. *Fertil. Steril.* Vol. **(68):** pp. 72-75.

Ijiland, M.M., Evers, J. L. H., Hoagland, H.J., Dunselman, G.A.J., and Lo, C. R. (1999). Endometrial wave direction switch and the outcome of in vitro fertilization. *Fertil. Steril.* Vol.**(71)**, no. **(3):** pp. 476-481.

Imoedemhe, D.A.G., and  Sgue, A.B. (1993). Subzonal multiple sperm injection in the treatment of previous failed human in vitro fertilization. *Fertil. Steril.* Vol. **(59):** pp.172-176.

Information for egg providers in an egg sharing arrangement (2002)(http://www.crmlondon.co.uk/PDFfiles/I-13-EggSharingProvider.pdf (Accessed on 14/05/06 at 12.11pm).

IVF-Infertility©http://www.ivf-infertility.com/ivf/hatching.php (accessed on 16/06/07 at 1.32pm).

Jansen, R.P. (2003).The effect of female age on the likelihood of a live birth from one in vitro fertilization treatment. *Med J Aust.*Vol.**(178)**;no.**(6)**:pp.258-261

Jean, M.L., Hermite, A., and Barriere, P. (1992). *Fertil. Steril.*Vol. **(57):** pp.591-596. In Edwards, R.G., and Brody, S.A. (1995). Principles and practice of assisted human reproduction, chapter**(9)**: Human fertilization in the laboratory: pp. 379.

Jennings, S. (1992). A lifeline offering support. Nurses' role in fertility counselling. *Prof. Nurse.***(7)**: pp.539-542.

Johnson, M. H., professor of reproductive sciences; member of HFEA et al (1997).The culture of unpaid and voluntary egg donation should be strengthened. *BMJ*.Vol. (**314**): pp. 1401.

Johnson, M.H. (1999). The medical ethics of paid egg sharing in the UK. *Hum.Reprod.* Vol. (**14**): pp.1912-1918.

Jones, G.M., Trounson, A.O., Gardner, D.K., Kausche, A., Lolatgis, N., and Wood, C. (1984).Evolution of a culture protocol for successful blastocyst development and pregnancy. *Hum.Reprod.* Vol (**13**): pp. 676-681.

Jones, H., Acosta, A., and Garcia, J. (1983). On the transfer on conceptuses from oocytes fertilized *in vitro*. *Fertil. Steril*.Vol. (**39**): pp. 241–243.

Jones, H.P., Lenz, R.W., Palevitz, B.A., and Cormier, M.J. (1990). Proc. Natl. Acad. Sci.USA,; Vol.(**77**):pp. 2722-2776. In Edwards, R.G., Brody, S.A.,(1995). Principles and practice of assisted human reproduction, Chapter (**8**): Oocyte growth, maturation and fertilization: pp. 309.

Jones, J.M., et al. Embryo culture, assessment, selection and transfer. ©www.who.int/reproductive-health/infertility/20.pdf ( Accessed on 24/09/06 at 3:43 pm)

Kallen, B., Finnstrom, O., Nygren, K.G., Olausson, P.O. (2005).In vitro fertilization (IVF) in Sweden : risk for congenital malformation after different IVF method. *Birth Defects Res A Clin. Mol. Teratol.* Vol. (**73**), no.(**3**): pp.162-169.

Kan, A.K.S., Abdalla, H.I., Ogunyemi, B.O., Korea, L., and Latarche, E. (1998). A survey of anonymous oocyte donors: demographics. *Hum. Reprod.* Vol. (**13**), no. (**10**): pp. 2762-2766.

Kannisto, P., Jorgensen, J., and Liedberg, F. (1992). Neotropeptides and ovulation. In Sjoberg, N.O., Hamberger, L., Janson, P.O., et al.(eds) Local regulation of ovarian function, *Parthenon, Carnforth* :pp. 201-208.

Karande, V., Morris, R., Chapman, C., Rinehart, J., and Gleicher, N. (1999); Impact of the "physician factor" on pregnancy rates in a large assisted reproductive technology program: do too many cooks spoil the broth? *Fertil. Steril*.Vol. (**71**): pp.1001–1009.

Kato, O., Kataksuka, R., and Asch, R.H. (1993); Transvaginal–transmyometrial embryo transfer: the Towako method; experience of 104 cases. *Fertil. Steril*.Vol. (**59**): pp. 51–53.

Kausche, A., Jones, G.M., Trounson, A.O., Figueiredo, F., MacLachlan, V., and Lolatgis, N. (2001).Sex ratio and birth weights of infants born as aresult of blastocyst transfer compared with early cleavage stage embryo transfer. *Fertil Steril*. Vol. (**76**), no. (**4**):pp. 688-693.

Kemeter, P., and Feichtinger, W. (1984). Pregnancy following in vitro fertilization and embryo transfer using pure human serum as culture and transfer medium. *Fertil. Steril.* Vol. (**41**): pp. 936- 937.

Kent-First, M.G., Kol, S., Muallem, A., Ofir, R., Manor, D., and Blazer, S. (1996). The incidence and possible relevance of Y-linked micro deletions in babies born after intracytoplasmic sperm injection and their infertile fathers. *Mol. Hum. Reprod.* Vol. (**2**): pp. 943–950.

Khan, A. K. S., Abdullah, H.I., and Gafar, A.H. (1999). Embryo transfer: ultrasound guided versus clinical touch *.Hum. Reprod.* Vol. (**14**): pp.1259-1261.

Kido, A., Togashi, K., Nakai, A., Kataoka, M.L., Koyama, T., and Fujii, S. (2005). Oral contraceptives and uterine peristalsis: *Evaluation with MRI, Journal of Magnetic Resonance Imaging.*Vol. (**22**), no. (**2**): pp. 265 – 270.

Kitawaki, J., Noguchi, T., and Amatsu, T. (1997). Expression of aromatase cytochrome P450 protein and messenger ribonucleic acid in human endometriotic and adenomyotic tissues but not in normal endometrium *.Biol.Reprod.*Vol. (**57**): pp. 514-519.

Kiyokawa, K., Masuda, H., Fuyuki, F., Kuseki, M., Uchida, N., Fukuda, T., Amemiya, K., and Suzuki. K. (2000).Three dimensional hysterosalpingo - contrast sonography (HyCoSy) as an out patient procedure for assess infertile women: a pilot study, Ultrasound *Obstet. Gynaecol.* Vol. (**16**): pp. 648-654.

Knutzen, V., Stratton, C. J., and Sher, G. (1992). Mock embryo transfer in early luteal phase, the cycle before in vitro fertilization and embryo transfer: a descriptive study *.Fertil. Steril.* Vol. (**57**): pp.156-162.

Kojima, K., Nomiyama, M., Kumamoto, T., Matsumoto, Y., and Iwasaka, T. (2001).Transvaginal ultrasound-guided embryo transfer improves pregnancy and implantation rates after IVF. *Hum Reprod.* Vol. (**16**).no. (**12**): pp. 2578-2582.

Koninckx, P. R., Meuleman, C., and Demeyere, S. (1991).Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrated endometriosis is associated with pelvic pain *.Fertil. Steril.* Vol. (**55**): pp.759-765.

Kovachev, E., Ganchev, Z.h., Cherneva, S., Zokhav, E., and Shperberg, A.(2005); Measurement of endometrial volume and endometrial thickness for the assessment of endometrial receptivity in assisted reproductive technique. *Akush Ginekol (Sofiia).*Vol.(**44**) no. (**2**): pp.27-33.Abstact in (Pub-Med).

Kovacs, G.T. (1991).What factors are important for successful embryo transfer after in vitro fertilization? *Hum.Reprod.* Vol. **(14):** pp. 590-592.

Kovacs, P. (2002). Unexplained Infertility and Repeated IVF, from Medscape Ob/Gyn & Women's Health©http://www.medscape.com/viewarticle/444956 (Accessed on 02/09/06 at 9:15am)

Kovacs, G. T., and Hewlett, D. (2004). IF at first you don't succeed, try, try, and try again: A successful birth after 37 cycles of assisted reproductive technology over 11 years. *Australian and Newzeland, J. of Obstet. And Gynecol.* Vol. **(44),** no. **(6):** pp. 580.

Krzyminska, U.B., Leung, P., O Neill, C., and Pike, I.L. (1992). *Lancet*; pp. 339-375. In Edward R.G., and Brody, S.A.(1995). Principles and practice of assisted human reproduction. Chapter **9:** Human fertilization in the laboratory: pp. 381.

Kuivasaari, P., Hippelainen, M., Anttilla, M., and Heinonen, S. (2005). Effect of endometriosis on IVF/ICSI outcome: stage 3, 4 endometriosis worsen cumulative pregnancy and live born rate. *Hum.Reprod.* Vol.**(20),** no. **(11):** pp. 3130-3135.

Kunz, G., Beil, D., Deininger, H., Wildt, L., and Leyendecker, G. (1996). The dynamics of rapid sperm transport through the female genital tract. Evidence from vaginal sonography of uterine peristalsis (VSUP) and hysterosalpingoscintigraphy (HSSG).*Hum.Reprod.*Vol.**(11):** pp. 627-632.

Kunz, G., and Leyendecker, G. (1996).Uterine peristalsis throughout the menstrual cycle: physiological and pathophysiological aspects. *Hum. Reprod.* Update, Vol.**(2),** no.**(4)** CD-ROM

Kunz, G., Beil, D., and Deininger, H. (1997).The uterine peristaltic pump .Normal and impeded sperm transport within the female genital tract .Adv. exp. *Med. Biol.* Vol. **(424):** pp. 267-277.

Kunz, G., Noe, M., Herbertz, M., and Leyendecker, G. (1998a). Uterine peristalsis during the follicular phase of the menstrual cycle .Effect of oestrogen, antiestrogen and oxytocin. *Hum. Reprod.* Vol. **(4):** pp. 647-654.

Kunz, G., Herbertz, M., Noe, M., and Leyendecker, G. (1998b). Sonographic evidence for the involvement of the utero-ovarian counter current system in the ovarian control of directed sperm transport .*Hum. Reprod. (Update)*; Vol. **(4):** pp. 667-672.

Kunz, G., Beil, D., Huppert, P., and Leyendecker, G. (2000).Structural abnormalities of the uterine wall in women with endometriosis and infertility visualized by vaginal sonography and magnetic resonance imaging .*Hum.Reprod.*Vol.**(15),** no. **(1):** pp. 76-82.

Kurzok, R., and Lieb, C.C. (1930). Biochemical studies of human semen: the action of semen on the human uterus. *Proc Soc Exp Biol Med.*Vol. (28): pp.272-298.

Lamont, J.A., and Anderson, L. (1993).The interaction between sexuality and infertility. *Can. J. Hum. Sex.*Vol. (2): pp.107- 112

Lane, D.E,,Vittinghoff, E., Croughan, M.S,, Cedars, M.I., and Fujimoto, V.Y., (2006).Gonadotropin stimulation demonstrates a ceiling effect on in vitro fertilization outcomes.*Fertil. Steril.* Vol. (85), no. (6): pp. 1708-1713

Langley, M.T., Marek, D.M., Gardner, D.K., Doody, K.M., and Doody, K.J. (2001).Extended embryo culture in human assisted reproduction treatments. *Hum. Reprod.*Vol. (16):pp. 902–908.

Lashen, H., Ledger, W., Lopez Bernal, A., Evans, B., and Barlow, D. (1998). Superovulation with a high gonadotropin dose for *in vitro* fertilization: is it effective? *J. Assist. Reprod. Genet.* Vol.(15): pp. 438–443

Lavery, S., Lawrie, H., Trew, G., and Winston, R. (1998). Age and basal follicle stimulating hormone as predictors of in vitro fertilisation outcome.*BJOG.*Vol.(105), no. (7): pp. 810.

Le Bourhis, C., Siffroi, J.P., McElreavey, K. (2000) Y chromosome microdeletions and germinal mosaicism in infertile males. *Mol. Hum. Reprod.*Vol.(6) : pp.688–693.

Lee, J. K. T., Gersell, D. J., Balfe, D.M., and Worthington, J.L. (1985). The uterus: in vitro MR-anatomic correlation of normal and abnormal specimens .*Radiology.* Vol. (156): pp. 175-179.

Leeton, J., Trounson, A., Jessup, D., and Wood, C. (1982).The technique for human embryo transfer .*Fertil. Steril.*Vol. (38): pp. 156-161.

Lenz, S., and Lauritzen, J.G. (1982). *Fertil. Steril;* Vol. (38): pp. 673 – 677. In Edwards, R.G., Brody, S.A., (1995). Principles and practice of assisted human reproduction, chapter 2: history and ethics of assisted human conception: pp. 21.

Leong, M., Leong, C., and Tucker, M. (1986). Ultrasound –assisted embryo transfer. *J. In Vitro Fertil. Embryo Transfer,* Vol. (3): pp. 383-385.

Lesny, P., Killick, S.R., and Tetlow, R.L. (1998a). Uterine junctional zone contractions during assisted reproduction cycles. *Hum. Reprod. Update,* Vol. (4): pp. 440–445.

Lesny, P., Killick, S.R., Tetlow, R.L, Robinson, J., and Maguiness, S.D. (1998b). Embryo transfer--can we learn anything new from the observation of junctional zone contractions? *Hum. Reprod.* Vol. (13): pp. 1540-1546.

Lesny, P., Killick, S.R., Robinson, J., Raven, G., and Maguiness, S.D. (1999a). Junctional zone contractions and embryo transfer: is it safe to use a tenaculum? *Hum. Reprod.*Vol. (14): pp. 2367–2370.

Lesny, P., Killick, S.R., Robinson, J., Titterington, J., and Maguiness, S.D. (1999b). Ectopic pregnancy after transmyometrial embryo transfer: case report. *Fertil. Steril.*Vol. (72): pp. 357–359.

Lessey, B. A., Killiam, A.S.P., and Metzger, D.A. (1988). Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J. Clin. Endocrinol. Metab.*, Vol. (67): pp. 334–340.

Levi, A.J., Segars, J.H., Miller, B. T., and Leondires, M. P. (2001). Endometrial cavity fluid is associated with poor ovarian response and increased cancellation rates in ART cycles*Hum.Reprod.* Vol.(16), no. (12): pp. 2610-2615.

Levitas, E., Lunenfeld, E., Har-Vardi, I., Albotiano, S., Sonin, Y., Hackmon-Ram R., and Potashnik G (2004). Blastocyst-stage embryo transfer in patients who failed to conceive in three or more day 2–3 embryo transfer cycles: a prospective, randomized study. *Fertil. Steril.* Vol.(81), no.(3): pp. 567-571.

Lewin, A ., Schenker, J.G., Avrech, O., Shapira, S., Safran, A., and Friedler, S. (1997).The role of uterine straightening by passive bladder distension before embryo transfer in IVF cycle *J Assisted Reprod .Genet.* Vol. (14): pp. 32-34.

Leyendecker, G., Kunz, G., and Wildt, L. (1996).Uterine hyper peristalsis and dysperistalsis as dysfunction of the mechanism of rapid sperm transport in patient with endometriosis and infertility. *Hum. Reprod.* Vol. (11): pp. 1542 - 1551.

Lin, Y.P., Cassidenti, D.L., and Chacon, R.R. (1995). Successful implantation of frozen sibling embryos is influenced by the outcome of the cycle from which they are derived. *Fertil. Steril.*Vol. (63): pp. 262.

Lopata, A., Johnson, W.I.H., Hoult, J., and Speris, A.I. (1980). *Fertil. Steril.*; (33) :117-120. In Edwards, R.G., and Brody, S.A., 1995: Principle and practice of assisted human reproduction, chapter (1): history and ethics of assisted human conception: pp. 26-35.

Lurie, S. (1992).The history of the diagnosis and treatment of ectopic pregnancy: a medical adventure. *Euro. J. Obst. Gynaecol. Reprod. Biol.* Vol. (9), no. 43(1): pp. 1-7.

Lutjen, P., Trounson, A., and Leeton, J. (1984). Establishment and maintenance of pregnancy using IVF and embryo donation in a patient with primary ovarian failure. *Nature.* (307):pp.174-175.

Lyons, E.A., Taylor, P.J., and Zheng, X. H. (1991). Characterisation of sub endometrial mymometrial contractions throughout the menstrual cycle in normal fertile woman .*Feril. Steril.*Vol. (55): pp. 771-775.

Magli, M.C., Gianaroli, L., Munne, S., and Ferrareti, A.P. (1998). Incidence of chromosomal abnormalities from a morphologically normal cohort of embryos in poor-prognosis patients. *Journal of Assisted Reproduction and Genetics.* Vol. (15): pp. 297–301.

Mansour, R., Aboulghar, M., and Serour, G. (1990). Dummy embryo transfers a technique that minimizes the problem of embryo transfer and improves the pregnancy rate in human IVF. *Fertil. Steril.*Vol. (54):pp. 678-681.

Mansour, R., Aboulghar, M., Serour, G., and Riad, R. (1991); Fluid accumulation of the uterine cavity before embryo transfer, a possible hindrance for implant. *J. Invitro fertilization and embryo transfer.*Vol. (8): pp. 157-159.

Mansour, R.T., Aboulghar, M.A., and Serour, G.I. (1994); Dummy embryo transfer using methylene blue dye .*Hum.Reprod.* Vol. (9): pp. 1257-1259.

Mansour, R.T., and Aboulghar, M.A. (2002).Optimizing the embryo transfer technique. *Hum. Reprod.* Vol. (17), no. (5): pp.1149-1152.

Marconi, G., Auge, L., and Oses, R. (1989). Does sexual intercourse improve pregnancy rate in gamete intrafallopian transfer? *Fertil. Steril.* , Vol. (51): pp. 357-359.

Marconi, G., Vilela, M., Bello, J., Diradorian, M., Quintana, R., and Sueldo, C. (2003). Endometrial lesions caused by catheters used for embryo transfer: a preliminary report.*Fertil.Steril.* Vol. (80), no. (2): pp. 363-367.

Marcoux, S., Maheux, R., and Berube, S. (1997). Laparoscopic surgery in infertile women with minimal or mild endometriosis. Canadian Collaborative Group on Endometriosis. *N. Engl. J. Med.*Vol. (337): pp. 217–222.

Marcus, S.F., and Brinsden, P.R. (1995).Analysis of the incidence and risk factors associated with ectopic pregnancy following in-vitro fertilization and embryo transfer. *Hum.Reprod.* Vol. (10): pp. 199-203.

Marek, D., Langley, M., Gardner, D.K., Confer, N., Doody, K.J., and Doody, K.M. (1999); Introduction of blastocyst culture and transfer for all patients in an in vitro fertilization program. *Fertil. Steril.* Vol. (72), no. (6): pp. 1035-1040.

Margalioth, E.J., Ben-Chetrit, A., Gal, M., Eldar-Geva, T. (2006). Investigation and treatment of repeated implantation failure following IVF-ET. *Hum. Reprod.* Vol.(21), no.(12):pp. 3036-3043.

Markus, M.1., Weghofer, A., Kogosowski, A., Zaki, K., and Feichtinger, W. (2003).A Prospective Randomized Multicenter Study to Evaluate the Best Day for Embryo Transfer: Does the Outcome Justify Prolonged Embryo Culture? *Journal of Assisted Reproduction and Genetics.* Vol. (**20**), no. (**2**): pp. 91-94.

Martikaiinen, H., Tiitinen. A., Tomas. C., Tapanainen, J., Orava, M., Tuomivaara, L., Vilska, S., Hyden-Granskog, C., and Hovatta, O. (2001). One versus two embryo transfer after IVF and ICSI: a randomized study. *Hum. Reprod.*Vol. (**16**):pp. 1900–1903.

Martinez, F., and Trounson, A. (1986). An analysis of factors associated with ectopic pregnancy in a human *in vitro* fertilization program. *Fertil. Steril.*Vol. (**45**): pp. 79–87.

Martinez, F., Coroleu, B., Parriego, M., Carreras, O., Belil, I., Parera, N., Hereter, L., Buxaderas, R., and Barri, P.N. (2001).Ultrasound-guided embryo transfer: immediate withdrawal of the catheter versus a 30 second wait, *Hum. Reprod.*Vol. (**16**): pp. 871-874

Martinez –Gaudio M., Yshida, T., and Bengtsson, L.P. (1973). Propagated and non-propagated myometrial contractions in normal menstrual cycles .*Am. J. Obstet. Gynaecol.* Vol. (**115**):pp. 107-111.

.Matorras, R., Mendoza, R., Expósito, A., and Rodriguez-Escudero, F.J. (2004). Influence of the time interval between embryo catheter loading and discharging on the success of IVF. *Hum. Reprod.*Vol.(**19**), no. (**9**): pp. 2027-2030.

McCarthy, S., Tauber, C., and Gore, J. (1986). Female pelvic anatomy, MR assessment of variation during the menstrual cycle and with the use of oral contraceptive. *Radiology.* ; Vol. (**160**): pp. 119-123.

McNamee, P.I. (1999). Embryo transfer techniques: is cervical mucus more important than we thought? In: *Postgraduate course 11th World Congress on In Vitro Fertilization and Human Genetics.* Sydney: pp. 38–48.

Meldrum, D., Chetkowski, R., Steingold, K.A., Deziagler, D., Cedars, M.I., and Hamilton, M. (1987). Evolution of a highly successful in vitro fertilization – embryo transfer program. *Fertil. Steril.*Vol. (**48**): pp. 86-93.

Meldrum, B., Marr, C., Stubbs, Wisot, A., Yeo, 1., and Hamilton, F. (1992). Impaired Uterine Receptivity in Infertile Women over Age 40 having Oocyte Donation and Correction with Increased Progesterone Replacement *Reproduction, Fertility and Development* Vol.(**4**), no. (**6**): pp. 689 – 693.

Mencken, J., Trussel, J., and Larsen, U. (1986). Age and infertility. (*Science*).Vol. (**233**): pp. 1389-1394.

Menezo, Y., Arnal, F., Humeau, C., Ducret, L., and Nicollet, B. (1989). Increased viscosity in transfer medium does not improve the pregnancy rates after embryo transfer. *Fertil. Steril.* Vol. **(52)**: pp. 680-682.

Menezo, Y.J., Chouteau, J., Torello, J., Girard, A., Veiga, A. (1999). Birth weight and sex ratio after transfer at the blastocyst stage in humans. *Fertil. Steril.* Vol. **(72)**: pp. 221–224.

Milki, A.A., Jun, S.H., Hinckley, M.D., Westphal, L.W., Giudice, L.C., and Behr, B (2003).Comparison of the sex ratio with blastocyst transfer and cleavage stage transfer .*J Assist Reprod Genet.* Vol.**(20)**,no.**(8)**:pp.323-326.

Mills, M.S., Eddowes, H.A., Cahill, D.J., Fahy, U.M., Abueid, M.I., McDermott, A., and Hull, M.G.(1992). A prospective controlled study of in vitro fertilization gamete intrafallopian transfer and intrauterine insemination combined with super ovulation .*Hum.Reprod.* Vol. **(7)**: pp. 490-494.

Mohr, L.R., and Trounson, A.O. (1982) Comparative ultra structure of hatched human, mouse and bovine blastocysts. *Reproduction, Fertility, and Development.*Vol. **(66)**: pp. 499–504.

Moncada, S., Palmer, R.M., and Higgs, E.A. (1991). Nitric oxide: physiology, path physiology, and pharmacology. *Pharmacol. Rev.* Vol. **(43)**: pp. 109–142.

Munne, S., Alikani, M., and Tomkin, G. (1995). Embryo morphology, developmental rate and maternal age are correlated with chromosome abnormality.*Fertil. Steril.*Vol. **(64)**:pp. 382–391.

Munne, S., and Cohen, J. (1998).Chromosome abnormalities in human embryos. *Hum. Reprod. Update;* Vol. **(4)**: pp. 842–855.

Naaktgeboren, N., Dieben, S., and Heijnsbroek, I. (1998). Embryo transfer easier said than done, abstract of 16th world congress on fertility and sterility and 54th *annual meeting of American society of reproductive medicine, San Francisco, USA.).*

Nabi, A., Awonuga, A., and Birch, H. (1997). Multiple attempts at embryo transfer does this affect In vitro-fertilization out come? *Hum. Reprod.*Vol. **(12)**: pp. 1188-1199.

Nakai, A., Togashi, K., Yamaoka, T., Ueda, H., Koyama, T., and Konishi, J. (2000).Uterine junctional zone on cine MR imaging (abstr). *Radiology.* Vol. **(217)**: pp. 276.

Nakai, A., Togashi, K., Ueda, H., Yamaoka, T., Fujii, S., and Konishi, J. (2001). Junctional zone on magnetic resonance imaging: continuous changes on ultrafast images. *J. Women's Imaging.* Vol. **(3)**: pp. 89-93.

Napolitano, R., and Fonttis, A.A. (2002). Pregnancy following difficult embryo transfer: a case report. *Reproductive Biomedicine.*Vol.(**5**), no. (**1**): pp. 47-49.

Natalija, V. (2005).Mobilization versus Bed Rest following Embryo Transfer (review) ©www.gfmer.ch/PGC_RH_2005/pdf/Mobilization_ET_R.pdf (Accessed on 09/09/06, at 8.10pm)

National Summary and Fertility Clinic Reports. Assisted Reproductive Technology Success Rates. (2001). ©www.cdc.gov/reproductivehealth/art.htm.Accessed on 26/09/06 at 5.02pm

Nazari, A., Askari, H.A., Check, J. H., and O Shaughnessy, A. (1993). Embryo transfer technique as a cause of ectopic pregnancy in in vitro fertilization. *Fertil. Steril.* Vol. (**60**): pp. 919-920.

Neithardt, A.B., Segars, J. H., Hennessy, S., James A.N., and McKeeby, J.L. (2005). Embryo afterloading: A refinement in embryo transfer technique that may increase clinical pregnancy.*Fertil.Steril.*Vol.(**83**), no.(**3**): pp.710-714.

Ng, S.C., Bangso, A., and Ratnam, S.S. (1988). Pregnancy after transfer of multiple sperm under the zona. *Lancet.* Vol. (**2**): pp. 790.

Ng, E.H., Chan, C.C., Tang, O.S., Yeung, W.S., and Ho, P.C.(2006).The role of endometrial and subendometrial Vascularity measured by three-diamential power Doppler ultrasound in the prediction of pregnancy during frozen thawed embryo transfer cycles.*Hum. Reprod.*Vol.(**21**),no.(**6**):pp1612-1617.

Nissole, M., and Donnez, M. (1997).Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities .*Ferti.Steril.* Vol. (**68**): pp. 585-596.

Noble, L, S., Takayama, K., and Zeitoun, K.M. (1997). Prostaglandin E2 stimulates aromatase expression in endometriosis–derived stromal cell .J .Clinic. *Endocrinol. Metabol.* Vol. (**82**): pp. 600-606.

Noe, M., Kunz, G., Herbertz, M., Mall G., and Leyendecker, G. (1999). The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: characterization of the endometrial–sub endometrial unit *Hum. Reprod.* Vol.(**14**), no. (**1**): pp. 190-197.

Noyes, N., Licciardi, F., Grifo, J., Krey, L., and Berkeley, A. (1999). In vitro fertilization outcome relative to embryo transfer difficulty: a novel approach to the forbidden cervix. *Fertil. Steril.*Vol. (**72**): pp. 261–265.

Oike, K., Obata, S., and Tagak, K. (1988). Observations of endometrial movements with transvaginal sonography. *J. Ultrasound Med.*Vol. (**7**): pp. 99.

Oike, K., Ishihara, K., and Kikuchi, S., (1990).A study on the endometrial movement and serum hormone level in connection with uterine contractions .*Acta Obstet. Gynecol. Jpn,* Vol.**(42)**: pp. 86-92.

Ovarian Aging and Infertility,©http://www.havingbabies.com/age-infertility-ovarian.html (Accessed on 19/8/2006 at 5.34pm).

Ozer, B. S., and Vermesh, M. (1999). Full term delivery following cryopreservation of human embryos for 7.5 years: Case Report: *Hum. Reprod.*Vol. **14**, no. **(6)**: pp. 1650 -1652.

Padilla, S.L., and Garcia, J.E. (1989). Effect of maternal age and number of in vitro fertilization procedures on pregnancy outcome . *Fertil. Steril.* Vol. **(52)**, no. **(2)**: pp. 270-273.

Palermo, G., Joris, H., and Devroey, P. (1992). Pregnancies after intracytoplasmic injection of a single spermatozoon into an oocyte. *Lancet.* Vol. **(340)**: pp. 17–18.

Palermo, G., Joris, H., and Derde, M.P. (1993). Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic sperm-injection. *Fertil. Steril.*Vol. **(59)**:pp. 826–835.

Palermo, G.D., Cohen, J., Alikani, M., Adler, A., and Rosenwaks, Z. (1995). Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility. *Fertil Steril.*Vol. **(63)**: pp. 1231–1240.

Pandian, Z., Bhattacharya, S., Ozturk, O., Serour, G.I., Templeton, A. (2004). Number of embryos for transfer following in vitro fertilization or Intracytoplasmic sperm injection. *Cochrane Database Syst Rev.* Vol.**(18)**, no.**(4)**:CD003416.

Papanikolaou, E.G., Haeseleer, E., Verheven, G., Vande Velde, H., Van Streirteghem, A.,Devroey, P., and Tournage, H.(2005**)**. Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture. A randomized prospective study. *Hum Reprod.* Vol.**(20)**, no. **(11)**:pp.3198-203.

Pehlivan T, Rubio C, Rodrigo L, Romero J, Remohi J, Simon C, Pellicer A. (2003). Impact of preimplantation genetic diagnosis on IVF outcome in implantation failure pateints. *Reprod Biomed.* Vol. **(6)**, no. **(2)**:pp.232-237

Pertoff, H., Laurent, T.C., and Laas, T. (1978). Density gradient prepared from colloidal silica particles coated by polyvinyl pyrrolidone .*Anal. Biochemistry.* Vol.**(88)**: pp. 271-272.

Pickard, R.S., Powell, P.H., and Zar, M.A.(1991);The effect of inhibitors of nitric oxide biosynthesis and cyclic GMP formation on nerve-evoked relaxation of human cavernosal smooth muscle. *Brit. J. Pharmacol.* Vol. **(104)**: pp. 755-759.

Pillai, M.C., and Meizel, S.J., (1991); *Exp, Zool.* **(257):** 384-393. In Edwards, R.G., Brody, S.A., (1995). Principles and practice of assisted human reproduction, chapter **(9):** Human fertilization in the laboratory: pp. 364.

Poindexter, A.N., Thompson, D.J., and Gibbons, W.E. (1986). Residual influenced by the number of attempts required to replace the embryos in failed embryo transfer. *Fertil. Steril.* Vol. **(46):** pp. 262–267.

Ponjaert-Kristoffersen, I., Bonduelle, M., Barnes, J., Nekkebroeck, J., Loft, A., Wennerholm, U.B., Tarlatzis, B.C., Peters, C., Hagberg, B.S., Berner, A., and Sutcliffe, A.G (2005).Intercollaborative study of Intracytoplasmic sperm injection –concieved in vitro fertilization-concieved and naturally conceived 5 years old child outcome. Cognitive and motor assessment. *Pediatrics.*Vol.(115),no.(3): pp.283-289

Pool, T. B., Ellsworth, L.R., and Garza, J.R. (1990). *Fertil. Steril.* In Edward RG, Brody, SA 1995. Principles and practice of assisted human reproduction. Chapter **(11):** implantation rates during IVF,GIFT and other forms of assisted conception: pp. 486.

Prapas, Y., Prapas, N., and Hatziparasidou, A. (1995). The echo guided embryo transfer maximize the In vitro-fertilization result *ACta. EUr . Fertil.* Vol. **(26):** pp.113-115.

Prapas, Y., Prapas, N., and Hatziparasidou, A. (2001).Ultrasound-guided embryo transfer maximizes the IVF results on day 3 and day 4 embryo transfer but has no impact on day 5. *Hum Reprod.*Vol. **(16):** pp. 1904–1908.

Prapas, Y. P., Panagiotidis, Y., Prapa, S., Vanderzwalmen, P., and Makedos, G. (2004). Cervical dilatation has a positive impact on the outcome of IVF in randomly assigned cases having two previous difficult embryo transfers. *Hum.Reprod.*Vol.(19), no. **(8):** pp.1791-1795.

Pritts, E.A., and Atwood, A.K. (2002).Luteal phase support in infertility treatment: a meta-analysis of the randomized trials. *Hum. Reprod.* Vol. **(17)**, no. **(9):** pp. 2287-229.

Pryor, J.L., Kent-First, M., Muallem, A., Van Bergen, A.H., Nolten, W.E.,Meisner, L., and Roberts K.P. (1997).Microdeletions in the Y chromosome of infertile men. *N. Engl. J. Med.* Vol. **(336):** pp. 534–539.

Pulkkinen, M.O. (1995).Oviductal function is critical for early human life *.Ann. Med.*Vol. **(27):** pp. 307-310.

Qasim, SM., Trias, A., and Karacan, M. (1996). Does the absence or presence of seminal fluid matter in patients undergoing ovulation induction with intrauterine insemination? *Hum. Reprod.* Vol. **(11):** pp. 1008-1010.

Queen, K., Dhabuwala, C. B., and Pierrepoint, C.G., (1981). The effect of the removal of the various accessory sex glands on the fertility of the male rats. *J. Reprod. Fertil.* Vol. **(62)**: pp. 423-426.

Randolph, J. R., Ying, Y.K., Maier, D.B., Schmidt, C.L., and Riddick, D.H. (1986).Comparison of real-time ultrasonography, hysterosalpingogrphy, laparoscopy/ hysteroscopy in the evaluation of uterine anomalies and tubal patency. *Fertil. Steril.*Vol. **(46)**: pp. 828-32.

Rao, Ch. V., and Ambarus, G. (1994). Novel regulation of pregnant human myometrial smooth muscle cell gap junctions by human chorionic gonadotrophin .*Endocrinology.* Vol. **(135)**: pp. 2772-2779.

Raziel, A., Friedle, S., and Schachter, M. (2002). Increased frequency of female partner chromosomal abnormality in patient with high order implantation failure after in vitro fertilization. *Fertil. Steril.*Vol. **(78)**, no. **(3)**: pp. 515-519.

Reproduction and Responsibility: The Regulation of New Biotechnologies, chapter **2**: Assisted reproduction
©http://www.bioethics.gov/reports/reproductionandresponsibility/chapter2.html
(Accessed on 23/08/2006) accessed at 5:10 pm.

Rezabek, K., Koryntova, D., and Zivny, J. (2001). Does bed rest after embryo transfer cause a worse outcome in vitro fertilization? *Ceska Gynekol.* Vol.**(66)**, no. **(3)**: pp. 175-178.

Richards, P.A., and Tiltman, A.J. (1995).Anatomical variation of the oestrogen receptor in normal myometrium. *Virchows Arch.* Vol. **(427)**: pp. 303–307.

Rienthaller, A., Riss, P., and Deutinger, J. (1986). Factors influencing successful in in-vitro fertilization and embryo transfer: a matched pair study. *Fertil. Steril.*Vol. **(46)**: pp. 511–513.

Rijnders, P.M., Van Os, H.C., and Jansen, C.A.M. (1998). Increased incidence of monozygotic twinning following the transfer of blastocysts in human IVF/ICSI. *Fertil. Steril.*; Vol.**(70)**: pp.S15–S16.

Robins, J., (1975). Midtrimester pregnancy termination by intramuscular injection of α -15 methyl analogue of PG F2α .*Am. J. Obst. Gynaecol.* Vol.**(123)**: pp. 623-631.

Rocha, A., Randel, RD., Broussard, J.R., Lim, J.M., Blair, R.M., Roussel, J.D., Godke, R.A., and Hansel, W. (1998). High environmental temperature and humidity decrease oocyte quality in Bos taurus but not in Bos indicus cows. *Theriogenology* .Vol. **(49)**: pp. 657–665.

Rosenlund, B., Sjoblom, P., and Hillensjo, T. (1996). Pregnancy outcome related to the site of embryo deposition in the uterus. *J. Assist. Reprod.Genet.* Vol. **(13)**: pp. 511–513.

Russell, J.A., and Leng, G. (1998). Sex parturition and motherhood without oxytocin? *(Review) Journal of endocrinology.* Vol. **(157)**: pp. 343-359.

Russell, J.B. (1998). The aetiology of ectopic pregnancy .*Clin. Obstet.Gynecol.* Vol. **(30)**:pp.181-190.

Saling, P.M., and Storey, B. T. (1979) *J. Cell Biol.*; **(83)**:544-555. In Edwards, R.G., and Brody, S.A.,(1995). Principles and practice of assisted reproduction chapter **(8)** .Oocyte growth, maturation, and fertilization: pp. 283-317.

Sallam, H.N., Farrag, F., and Ezzeldin, A. (2000).Vigorous flushing of the cervical canal prior to embryo transfer a prospective randomised study. *Fertil.Steril.*Vol.**(74)**, no. **(l)**: pp. 203.

Sallam, H. N., Agameya, A. F., Rahman, A. F., Ezzeldin, F., and Sallam, A. N. (2002).Ultrasound measurement of the utero cervical angle before embryo transfer: a prospective controlled study. *Hum.Reprod.* Vol.**(17)**, no. **(7)**:pp. 1767-1772.

Salumets, A., Hyden-Granskog, C., Makinen, S., Suikkari, A., Tiitinen, A., and Tuuri, T. (2003) Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures. *Hum. Reprod.* Vol. **(18)**: pp. 821–825.

Sampson, J.A. (1927). Peritoneal endometriosis due to menstrual dissemination of endometrial tissue in to the peritoneal cavity .*Am .J .Obstet .Gynaecol.* Vol. **(14)**: pp. 422-429.

Samuelsson, B. (2003).The prostaglandins. *Angewandte Chemie International Edition in English.*Vol. **(4)**, no. **(5)**: pp. 410 – 416.

Saulsberry, S.B., and Pohlaus, M.F. (1992) Assessment and initial management of infertility. *J. Am. Acad. Nurse Pract.* Vol. **(4)**: pp. 53-57.

Schlegel ,P. N., and Girardi, S. K. (1997). *In Vitro* Fertilization for Male Factor Infertility.*Journal of Clinical Endocrinology and Metabolism;* Vol. **82**, no. **(3)**:pp.709-716©http://jcem.endojournals.org/cgi/content/full/82/3/709 Accessed on 12/07/06, at3:42p

Schoolcraft, W.B., and Gardner, D.K. (2000). Blastocyst culture and transfer increases the efficiency of oocyte donation. *Fertil. Steril.*Vol. **(74)**:pp. 482–486

Schoolcraft, W.B., Surrey, E.S., and Gardner, D.K. (2001); Embryo transfer: techniques and variables affecting success.*Ferti. Steril.*Vol. **(76)**: pp. 863-870.

Schwärzler, P., Zech, H., Auer, M., Pfau, K., Göbel, G., Vanderzwalmen, P., and Nicolas, Z. (2004). Pregnancy outcome after blastocyst transfer as compared to early cleavage stage embryo transfer. *Hum. Reprod.*Vol. **(19)**, no. **(9)**: pp. 2097-2102.

Scoutt, L. M., Flynn, S.D., and Luthrringer, D.J. (1991).Junctional zone of the uterus: Correlation of MR imaging and histological examination of hysterectomy specimens. *Radiology.* Vol. **(179)**: pp. 403-407.

Scoutt, L. (2002).Embryological strategies for overcoming recurrent assisted reproductive technology treatment failure, *Hum. Fertil.* Vol. (5): pp. 206–214.

Selick, C.E., Hofmann, G.E., and Albano, C. (1995). Embryo quality and pregnancy potential of fresh compared with frozen embryos – is freezing detrimental to high quality embryos? *Hum. Reprod.* Vol. **(10)**: pp. 392–395.

Serhal, P., Ranieri,, D. M., Iffat, K., and Rami, A. W. (2003). Cervical dilatation with hygroscopic rods prior to ovarian stimulation facilitates embryo transfer. *Hum.Reprod.*Vol.**(18)**, no. **(12)**: pp. 2618-2620.

Shabonowitz, R. B., and O Rand, M. G. (1988).Characterization of the human zona pallucida from fertilized and unfertilized eggs. *Journal of reproductive medicine.* Vol. **(82)**: pp. 151-161.

Shaker, A.G. (1993); Assessment of embryo transfer after in vitro fertilization effect of Glyceryl nitrate. *Hum. Reprod.*Vol.**(8)**: pp. 1426-1428.

Sharara, F.I., Scott, R.T., Marut, E.L., and Queen an, J.T. (1996). In-vitro fertilization outcome in women with hydrosalpinx. *Hum. Reprod.*Vol. **(11)**: pp.526–530.

Sharara, F. I., and McClamrock, H. D. (1997); endometrial fluid collection in women with hydrosalpinx after human chorionic gonadotrophin administration: a report of two cases and implications for management. *Hum. Reprod.* Vol.**(12)**, no. **(12)**: pp. 2816–2819.

Sharif, K., Afnan, M., and Lenton, W. (1995). Mock embryo transfer with full bladder immediately before the real transfer for IVF treatment: The Birmingham experience of 113 case .*Hum. Reprod.* Vol. **(10)**: pp. 1715-1718.

Sharif, K., Afnan, M., and Lenton, W. (1996).Transmyometrial embryo transfer after difficult immediate mock transcervical transfer. *Fertil. Steril.*Vol. **(65)**: pp. 1071–1074.

Sharif, K., Afnan, M., Lashen, H., Elgendy, M., Morgan, C., and Sinclair, L. (1998). Is bed rest following embryo transfer necessary? *Fertil. Steril.*Vol. **(69)**: pp. 478-481.

Sharif, K., Elgendy, M., Lashen, H., and Afnan, M. (1998). Age and basal follicle stimulating hormone as predictors of in vitro fertilisation outcome. *BJOG*. Vol. **(105)**: pp.107.

Sher, G., Feinman, M., and Zouves, C. (1994). Immunology: High fecundity rates following in– vitro fertilization and embryo transfer in antiphospholipid antibody seropositive women treated with heparin and aspirin et al *Hum.Reprod.* Vol. **(9)**, no. **(12)**: pp. 2278-83.

Sherman, J.K. (1963).Improved method of preservation of human sperm by freezing and freeing – drying. *Fertil. Steril.*Vol. **(14)**: pp. 49.

Shettles, L.B. (1979) *Am .J. Obstet. Gynecol*; **(133)**: 845. In Edwards, R.G., and Brody, S.A., 1995: Principle and practice of assisted human reproduction, chapter **(1)**: history and ethics of assisted human conception: pp. 26-35.

Sills, E.S., Moomjy, M., Zaninovic, N., Veeck, L.L., McGee, M., Palermo, G.D., and Rosenwaks, Z. (2000). Human zona pellucida micromanipulation and monozygotic twinning frequency after IVF. *Hum. Reprod.*; Vol. **(15)**: pp.890–895.

Sim, J.A., Jones, D., Butler, L., and Musher, S.J. (1993). Effect of Hydrosalpinx on the outcome in invitro fertilization presented at the 49th annual meeting of the American fertility society. *American fertility society program supplement* S95.

Sinclair, L., Morgan, C., Lashen, H., Afnan, M., and Sharif, K. (1998) . Nurses performing embryo transfer: the development and results of the Birmingham experience. *Hum. Reprod.* Vol.**(13)**, no. **(3)**: pp. 699-702.

Slikke H.V., & Professor Corson S. L. (2002). Embryo Transfer Techniques and Pregnancy Outcomes. OBGYN.net Conference Coverage Advances in Infertility.
http://www.obgyn.net/displaytranscript.asp?page=/avtranscripts/AIT2002_Corson
(Accessed on 14/07/06 at 3.24pm)

Slotnick, R.N., and Ortega, J.E. (1996). Monoamniotic twinning and zona manipulation: a survey of U.S. IVF centres correlating zona manipulation procedures and high-risk twinning frequency. *Journal of Assisted Reproduction and Genetics.* Vol. **(13)**: pp.381–385.

Smith, L.C. (1993). Membrane and intracellular effects of ultraviolet irradiation with Hoechst 33342 on bovine secondary oocytes matured in vitro. *J Reprod Fertil* .Vol. **(99)**: pp. 39–44.

Snijder, M., Bouchard, P., and De Brux, J. (1992); Use of immune cytochemistry of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause .*J.Reprod. Fertil.* Vol. **(94)**: pp. 363-367.

Spandorfer, S.D., Avrech, O.M., Colombero, L.T., Palermo, G.D., and Rosenwaks, Z. (1998) .Effect of parental age on fertilization and pregnancy characteristics in couples treated by intracytoplasmic sperm injection. *Hum. Reprod.*Vol. **(13)**: pp. 334-338.

Staessen, C., Camus, M., Khan, I., Van Waesberghe, L., Wisanto, A., Devroey, P., and Van Steirteghem, A.C. (1989).An 18 month survey of infertility treatment by in vitro fertilization gamete and zygote intra fallopian transfer and replacement of frozen thawed embryos. *J. In Vitro Fertil. embryo Transfer.*Vol.**(6)** : pp. 22-29.

Staessen, C., Camus, M., Bollen, N., Devroey, P., and Van Steirteghem, A.C. (1992). The relation ship between embryo quality and the occurrence of multiple pregnancies. *Fertil. Steril.*Vol. **(57)**: pp. 626-630.

Staessen, C., Janssenswillen, C., Van den Abbeel , E., Devroey , P., and Van Steirteghem, A.C. (1993).Avoidance of triplet pregnancies by elective transfer of two good quality embryos . *Hum. Reprod.*Vol. **(8)**: pp. 1650- 1653.

Staessen, C., Janssen Swillen  C., Devroey, P., and Van Steirteghem, A.C. (1993). Cytogenitic and Morphological observation of single pronucleated human oocyte after in vitro fertilization .*Hum. Reprod.* Vol. **(8)**: pp. 221-223.

Stein, Z. A. (1985). Woman's age, childbearing and child rearing. *Am J. Epidemiol*; Vol. **(121)**:pp. 327-342.

Steptoe, P.C., and Edwards, R.G. (1970). Laparoscopic recovery of pre – ovulatory human oocytes after priming of ovaries with gonadotrophin. *Lancet*; Vol. **(1)**: pp. 683-689.

Steptoe, P.C., and Edwards, R.G. (1976).Reimplantation of human embryo with subsequent tubal pregnancy. *Lancet.* Vol. **(1)**: pp. 880 -882.

Steptoe, P. C., and Edwards, R. G. (1978). Birth after the re implantation of a human embryo. *Lancet.* Vol. **(2)**: pp. 366.

Stewart, C.J.R., Farquharson, M.A., and Foulis, A.K. (1992);The distribution and possible function of gamma interferon immunreactive cells in normal endometrium and myometrium .*Virchows Arch A. Pathol A. nat.*Vol.**(420)**: pp. 419-424.

Strandell, A., Waldenstrom, U., Nilsson, L., and Hamberger, L. (1994). Hydrosalpinx reduce invitro fertilization embryo transfer pregnancy rate .*Hum. Reprod.* Vol. **(9)**: pp. 861-863.

Strandell, A., Sjögren, A., and Bentin-Ley, U.(1998). Hydrosalpinx fluid does not adversely affect the normal development of human embryos and implantation *in vitro. Hum. Reprod.*Vol. **(13)**: pp. 2921–2925.

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Strandell, A., Lindhard, A., Waldenstrom, U., Thorburn, J., Janson, P.O., and Hamberger, L. (1999).Hydrosalpinx and IVF outcome: a prospective randomized multicentre trial in Scandinavia on salpingectomy prior to IVF. *Hum. Reprod.* Vol. (**14**), no. (**11**):pp.2762-2769.

Strickler, R. C., Christianson, C., and Crane, J.P. (1985). Ultrasound guidance for human embryo transfer .*Fertil. Steril.*Vol. (**43**): pp. 54 -61.

Surrey, E.S., and Schoolcraft, W.B. (2001); Laparoscopic management of hydrosalpinx before in vitro fertilization – embryo transfer .salpingectomy versus proximal tubal occlusion. *Fertil. Steril.*Vol.(**75**), no. (**3**): pp. 612-7.

Tabibzadeh, S. S., Satyaswaroop, P.G., and Rao, P.N. (1988). Anti-prolifretive effect of interferon -gamma in human endometrial epithelial cells in vitro: potential local growth modulatory role in endometrium .*J. clin. Endocrinal . Metabol.* Vol. (**67**):pp. 131-138.

Tait, R.L. (1884). Five cases of extra uterine pregnancy operated up on at the time of rupture. *Br. Med. J.* Vol. (**1**): pp. 1250-1251.

Takahashi, K., Nagata, H., and Kitao, M. (1989).Clinical usefulness of determination of oestradiol level in the menstrual blood for patients with endometriosis .*Acta Obstet. Gynecol .Jpn.* Vol. (**41**): pp.1849-1850.

Templeton, A. Morris, K.J., and Parslow, W. (1996). Factors that affect outcome of in vitro fertilization treatment .*Lancet.* Vol. (**23**), no. (**348**): pp. 1402-1406.

Templeton, A., and Joan, and K. M. (1998). Reducing the Risk of Multiple Births by Transfer of Two Embryos after in Vitro Fertilization. *The New England journal of medicine* .Vol. **339**, no. (**9**): pp. 573-577.

Tesarik, J., Junca, A.M., Hazout, A., Aubriot, F.X., Nathan, C., Cohen-Bacrie, P., and Dumont-Hassan, M.(2000).Embryos with high implantation potential after intracytoplasmic sperm injection can be recognized by a simple, non-invasive examination of pronuclear morphology. *Hum. Reprod.* Vol. (**15**), no. (**6**): pp. 1396-1399.

Testart, J., Lassalle, B., and Belaisch - Allart, J. (1986). High pregnancy rate after early human embryo freezing. *Fertil. Steril.*Vol. (**46**): pp. 268 – 272.

Tetlow, R., Richmond, I., Greenman, J. (1997). Morphometric analysis of the uterine junctional zone as seen by ultrasound. *Acta Obstet. Gynecol. Scand.*Vol. (**76**): pp. 29.

The National Gamete Donation Trust. © http://www.ngdt.co.uk/( Accessed on 2/07/06 at 11.12am).

The Regulation of New Biotechnologies, chapter (2): Assisted reproduction) www.bioethics.gov/reports/reproductionandresponsibility/chapter2.html-243k (Accessed on 23/ 8/2006 at 5.10 pm)].

Thum, M., Gafar, A., Wren, M., Faris, R., Ogunyemi, B., Korea, L., Scott, L., and Abdulla, H.I. (2003). Does egg-sharing compromise the chance of donors or recipients achieving a live birth? *Hum. Reprod.* Vol.(18), no. (11):pp. 2363-2367.

Tietze, C. (1957). Reproductive span and rate of reproduction among Hutterite women. *Fertil. Steril.* Vol. (8):pp. 89–97.

Togashi, K., Kawakami, S., and Kimura, I. (1993). Sustained Uterine Contractions a cause of hypo intense Myometrial Bulging –*Radiology*; Vol. (187): pp. 707-710.

Tomas, C., Tikkinen, K., Tuomivaara, L., Tapanainen, S. J., and Martikaiinen, H. (2002). The degree of difficulty of embryo transfer is an independent factor for predicting pregnancy .*Hum. Reprod.* Vol. (177), no. (10):pp. 2632-2635.

Toner, J.P., Brzyski, R.G., and Oehninger, S. (1991a). Combined impact of the number of pre-ovulatory oocytes and cryopreservation on IVF outcome. *Hum. Reprod.* Vol. (6): pp. 284–289.

Toshimichi, O., Kuninori, M., Sachico, N., and Yukihiro, N. (2002).Changes in endometrial wave like movement in accordance with the phases of menstrual cycle .*J. of Obstet. and Gynaecol .research* .Vol. (28), no. (3): pp. 176..

Toya, M., Saito, H., Ohta, N., Saito, T., and Kaneko, T.M. (2000). Moderate and sever endometriosis is associated with severe alteration in the cell cycle of granulosa cells in patients undergoing in vitro fertilization and embryo transfer.*Fertil.Steril.*Vol.(73), no.(2): pp. 344-350.

Tremellan, K.P., Valbuana, D., Landeras, J., Bellesteros, A., Martinez, J., Mendoza, S., Norman, R.J., Robertson, S.A., and Simon, C. (2000). The effect of intercourse on pregnancy rate during assisted human reproduction .*Hum. Reprod.* Vol. (15), no. (12): pp. 2653-2658.

Trounson, A., and Mohr, L. (1983).Human pregnancy following cryopreservation, thawing and transfer of an eight cell embryo, *Nature*. Vol. (305): pp. 707.

Tucker, M.J., Bishop, F.M., and Cohen, J. (1991).*Hum. Reprod.* ; (6): pp.676-681. In Edwards, R.G., Brody, S.A.(1995).Principles and practice of assisted human reproduction ,chapter (9); Human fertilization in the laboratory: pp. 379.

Tur – Kaspa, I., Yuval, Y., and Bider, D. (1998). Difficult or repeated embryo transfers do not adversely affect in vitro fertilization pregnancy rate or outcome. *Hum. Reprod.* Vol. (13): pp. 2452-2455.

Turnbull, L. W., Rice, C. F., and Horsman, A. (1994). Magnetic resonance imaging and transvaginal ultrasound of the uterus, prior to embryo transfer. *Hum. Reprod;* Vol. **(9):** pp. 2438-2443.

Turnbull, L., W., Manton, D. J., Horsman, A., and Killick, S. R. (1995).Magnetic resonance imaging changes in uterine zonal anatomy during a conception cycle .*Br. J. Obstet. and Gynaecol.* Vol. **(102):** pp. 330-331.

Van Dromme, J., Chasse, E., and Lejeune, B. (1995). Hydrosalpinges in in-vitro fertilization: an unfavourable prognostic feature. *Hum. Reprod.* Vol. **(10):** pp.576–579.

Van Os, H.C., Alberda, A.T., Janssen- Caspers, H.A., Leerentveld R.A., Scholtes, M.C., Zeilmaker, G.H. (1989) . The influence of the interval between in vitro fertilization and embryo transfer and some other variables on treatment outcome. *Fertil. Steril.* Vol. **(51):** pp.360–362.

Van Steirteghem, A.C., Van den Abeel, E., Camus, M., and Devroey, P. (1992). Cryopreservation of human embryos. *Balliere's Clin Obstet Gynaecol.* Vol. **(6):** pp. 313-325.

Van Steirteghem, A.C.(1993a). Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. *Hum. Reprod.* Vol. **(8):** pp. 1055–1060.

Van Steirteghem, A.C. (1993b). High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod.* Vol. **(8):** pp. 1061–1066.

Van Steirteghem, A.C., and Van den Abbeel, E. (1993). Freezing of embryos: early versus late stages *J.Assist Reprod Genet.*Vol. **(10):** pp. 185- 186.

Van Steirteghem, A.C., Liu, J., Joris, H., Nagy, Z., Janssenswillen, C., Tournaye, H., Derde M.P., Van Assche A., and Devroey, P. (1993) Higher success rates by intracytoplasmic sperm injection than by subzonal insemination.Reprt of a second series of 300 consecutive treatment cycles. *Hum Reprod.*Vol. **(8):** pp. 1055-1060.

Van Steirteghem, A.C., Van der Elst, J., Van den Abbeel, E., Joris, H., Camus, M., and Devroey, P., (1994a) Cryopreservation of supernumerary multicellular human embryos obtained after intracytoplasmic sperm injection. *Fertil Steril.*Vol. **(62):**pp. 775-780.

Van Steirteghem, A.C., Nagy, Z., Liu, J., Joris, H., Verheyen, G., Smitz, J., Tournaye, H., Liebaers, I., and Devroey, P., (1994b) Intracytoplasmic Sperm injection.*Bailliere's Clin Obstet Gynaecol.* Vol. **(80):** pp. 85-93.

Veeck, L. (1987). Oocyte assessment and biological performance *Ann .N. Y. Acad. Sci. Vol.* **(541):** pp. 259-274.

Vidaeff, A.C., Racowsky, C., and Rayburn, W.F. (2000). Blastocyst transfer in human in vitro fertilization. A solution to the multiple pregnancy epidemics. *J. Reprod. Med.*Vol. **(45):**pp. 529–539.

Visser, D.S., Fourie, F., and Kruger, H.F. (1993). Multiple attempts at embryo transfer: effect on pregnancy outcome in an in vitro fertilization and embryo transfer program. *J. Assist. Reprod. Genet.*Vol. **(10):** pp. 37–43.

Wardle, P., McLaughlin, E., McDermott, E., Mitchell, J.D., Ray, B.D., and Hull, M.G.R. (1985).Endometriosis and ovulatory disorder reduced fertilization in vitro compared with tubal and unexplained infertility. *Lancet.* Vol. **(11):** pp. 236-239.

Webb, P., Lopez, G. N., Uht, R. M., and Kusher, P. J. (1995). Tamoxifen activation of the oestrogen receptor / AP-1 pathway: potential origin for the cell specific oestrogen-like effect of antiestrogen *.Mol .Endocrinol.* Vol. **(9):** pp. 443-456.

Webster, J. (1986). Embryo replacement. In Fishel , S., and Symonds , EM., (eds) IVF : past, present , and future . *IRL, press Oxford* : pp. 127-134.

Welker, B.G., Gembruch, U., Diedrich, K., Al-Hasani, S., and Krebs, D. (1989). Transvaginal ultrasonography of the endometrium during ovum pickup in stimulated cycles for *in vitro* fertilization. *J. Ultrasound Med.* Vol. **(8):** pp. 549–553.

Wenstrom, K.D., Syrop, C.H., Hammit, D.G., and VanVoorhis, B.J. (1993). Increased risk of monochorionic twinning associated with assisted reproduction. *Fertil. Steril.* Vol.**(60):** pp.510–514.

Werth, R., and Grusdew, W.(1898); Untersuchungen uber die Entwicklung undMorphologie der menschlichen Uterusmuskula *tur .Arch .Gynaecol.* Vol. **(55):**pp. 325-409. In Noe et al. (1999). The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: characterization of the endometrial–sub endometrial unit *Hum. Reprod.* Vol. **(14)**, no. **(1):** pp. 190-197.

Wetzstein, R. (1965). Der Uterusmuskel: Morphologie. *Arch. Gynecol.* Vol. **(202):** pp. 1–13. In Noe et al. (1999). The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: characterization of the endometrial–sub endometrial unit *Hum. Reprod.* Vol. **(14)**, no. **(1):** pp. 190-197.

White, D., and Aitkin, R.J. (1989). Relationship between calcium, cyclic AMP, ATP and intracyclic pH and the capacity of hamster spermatozoa to express

hyperactivated motility. *Gamete Res,* Vol. **(22):** pp.163-177.

Whittingham, and Wilmut (1972). In Fishel, S., and Symonds, M. E. (1986). IN VITRO FERTILIZATION; Past, Present, Future; Historical perspective: pp.12.

Wiczyk,H.P., Janus, C.L., and Richard, C.J. (1988). Comparison of magnetic resonance imaging and ultrasound in evaluating follicular and endometrial development throughout the normal cycle. *Fertil. Steril.* Vol. **(49):** pp. 969-972.

Wildt, L., Kissler, S., Licht, P., and Becker, W. (1998); Sperm transport in the human female genital tract and its modulation by oxytocin as assessed by hysterosalpingoscintigraphy, hysterotonography, electrohysterography and Doppler sonography. *Hum Reprod.* Vol.**(4),** no. **(5):** pp. 655–66.

Wiley, L.M. (1987). Development of the blastocyst: role of cell polarity in cavitation and cell differentiation. In: Bavister B.D., ed. *The mammalian preimplantation embryo: regulation of growth and differentiation in vitro. New York, Plenum Press:* pp.65–93.

William, M., Hill, C.S., and Scudamore, I. (1993). Sperm normal distribution with in the human fallopian tube and ovulation .*Hum. Reprod.*Vol. **(8):**pp. 2019- 2026.

Williams, S.C., Oehninger, S., Gibbons, W.E., Van Cleave, W.C., and Muasher, S.J.(2001) Delaying the initiation of progesterone supplementation results in decreased pregnancy rates after in vitro fertilization: a randomized, prospective study. *Fertil.Steril.* Vol. **(76),**no.**(6):**pp.1140-3

Willms, A.B., Brown ED, Kettritz, U.I., Kuller, J.A., and Semelka, R.C. (1995). Anatomic changes in the pelvis after uncomplicated vaginal delivery: evaluation with serial MR imaging. *Radiology.* Vol. **(195):** pp. 91–94.

Wilson, M., Hartke, K., Kiehl, M., Rodgers, J., Brabec, C., and Lyles, R. (2002). Integration of blastocyst transfer to all patients. *Fertil. Steril.* Vol. **(77):** pp. 693–696.

Wingfield, M., Macpherson, A., Healy, D. L., and Roger, P.A.W. (1995).Cell proliferation is increased in the endometrium of women with endometriosis .*Fertil. Steril.*Vol. **(64):** pp. 340-346.

Winston, N.J., Braude, P.R., and Pickering, S.J., (1991); *Hum.Reprod.* **(6):** pp.17-24. In Edwards, R.G., Brody, S.A.(1995).Principles and practice of assisted human reproduction, Chapter **(10)** : the human embryo in vivo and in vitro : pp. 442.

Wisanto, A., Camus, M., and Janssen, R. (1989).Performance of different embryo transfer catheter in a human in vitro-fertilization program .*Fertil. Steril.* Vol. **(52):** pp. 79-84.

Wood, C. (1981).A clinical assessment of nine pregnancies obtained by in vitro fertilization and embryo transfer. *Fertil. Steril.*Vol. **(35)**:pp. 502-508.

Wood, C., McMaster, R., Rennie, G., Trounson, A., and Leeton, J. (1985).Factors influencing pregnancy rates following in vitro fertilization and embryo transfer. *Fertil. Steril.*Vol. **(43)**: pp. 245–250.

Wood, E.G., Batzer, F. R., Go, K. J., Guttmann, J.N., and Carson, S. L.(2000).Ultrasound-guided soft catheter embryo transfers will improve pregnancy rates in in-vitro fertilization. *Hum. Reprod.* Vol. **(15)**, no. **(1)**: pp. 107-112.

Woolcott, R., and Stanger, J., (1994).The fluid dynamics of injection: Variables as they relate to the Trans vaginal gamete intra fallopian transfer and tubal embryo transfer. *Hum. Reprod.*Vol. **(9)**: pp.1670-1672.

Woolcott, R. and Stanger, J. (1997). Potentially important variables identified by transvaginal ultrasound-guided embryo transfer. *Hum. Reprod.*Vol.**(12)** no. **(5)**: pp.963–966.

Woolcott, R., and Stanger, J.(1998). Ultrasound tracking of the movement of embryo – associated air bubble on standing after transfer. *Hum. Reprod.* Vol. **(13)**: pp. 2107-2109.

Yallampalli, C., Garfield, R.E., and Byam-Smith, M. (1993). Nitric oxide inhibit uterine contractility during pregnancy but not delivery .*Endocrinology.* Vol. **(133)**: pp. 1899-1902.

Yallampalli, C., Dong, Y.L., and Gangula, P.R. (1998). Role and regulation of nitric oxide in the uterus during pregnancy and parturition. *J. Soc. Gynecol. Invest.*Vol. **(5)**: pp. 58–67.

Yang, D., Mohammed, A.M., Moza, A.L., Bader, S.D., Mariam, A.F., and Talha, A.S.(1996). Intracytoplasmic sperm injection improving embryo quality: Comparison of the sibling oocytes of Non-Male-Factor couples. *Journal of Assisted Reproduction and Genetics* Publisher: Springer Science Business Vol. **(13)**, no. **(4)**: pp.351 - 355.

Zech, H., Stecher, A., and Riedler, I. (1997); High implantation rate with a fast and a traumatic embryo transfer technique. *Hum. Reprod.* Vol.**(12)** (Abstract Bk 1): pp. 156.

Zeyneloglu, H.b., Arici, A., and Olive, D.L. (1998). Adverse effects of hydrosalpinx on pregnancy rates after in vitro fertilization –embryo transfer. *Fertil. Steril.* Vol.**(70)**, no. **(3)**: pp.492-499.

Zhao, K., Kuperman, L., Geimonen, E., and Anderson, J. (1996). Progestin represses human connexion 43 gene expression similarly in primary cultures of myometrial and uterine leiomyoma cells .*Biol. Reprod.* Vol. **(54)**: pp. 607-615.

Zingg, H., Rosen, F., and Chu, K. (1995). Oxytocin and oxytocin receptor gene expression in the uterus. *Recen , Progesterone hormone receptor, Research.* Vol.**(50)**: pp. 255-273.