Human embryos from overweight and obese women display phenotypic and metabolic abnormalities

Running title – Developmental disruption in embryos from overweight women

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Abstract;

Study question: Is the developmental timing and metabolic regulation disrupted in embryos from overweight and obese women?

Summary answer: Human oocytes from women presenting for fertility treatment with a body mass index (BMI) exceeding 25kg/m^2 are smaller than those from women of healthy weight, yet post fertilization they reach the morula stage faster. Moreover, the blastocysts from overweight women show reduced glucose consumption and contain elevated levels of endogenous triglyceride.

What is known already: Female overweight and obesity (OW/OB) is associated with infertility. Moreover, being overweight or obese around conception may have significant consequences for the unborn child, since there are widely acknowledged links between events occurring during early development and the incidence of a number of adult disorders.

Study design, size, duration: We have performed a retrospective, observational analysis of oocyte size and the subsequent developmental kinetics of 218 oocytes from 29 consecutive women attending for ICSI treatment and related time to reach key developmental stages to maternal bodyweight. In addition we have measured non-invasively the metabolic activity of 150 IVF/ ICSI embryos from a further 29 consecutive women who donated their surplus embryos to research, and related the data retrospectively to their BMI.

Participants/materials, setting, methods: In a clinical IVF setting, we compared oocyte morphology and developmental kinetics of supernumerary embryos collected from overweight and obese women, with a body mass index (BMI) in excess of 25kg/m^2 to those from women of healthy weight. Full informed consent was obtained. A Primovision Time Lapse system was used to measure developmental kinetics and

the non-invasive COnsumption/RElese (CORE) of glucose, pyruvate, amino acids, and lactate were measured on spent droplets of culture medium. Total triglyceride within individual embryos was also determined.

Main results and the role of chance: Human oocytes from overweight women are smaller (R^2 =-0.45; p=0.001) and less likely to complete development post-fertilization (p<0.001). Those embryos that do develop reach the morula stage faster than embryos from women of a BMI <25 kg/m^2 (<0.001) and the resulting blastocyst contain fewer cells; notably in the trophectoderm (p=0.01). The resulting blastocysts have reduced glucose consumption (R^2=-0.61; p=0.001), modified amino acid metabolism and increased levels of endogenous triglyceride (t=4.11, p<0.001). Our data further indicate that these differences are independent of male BMI.

Limitations, reasons for caution: Although statistical power has been achieved, this is a retrospective study and relatively small due to the scarcity of human embryos available for research. Consequently, sub analysis of overweight and obese was not possible based on the sample size. The analysis has been performed on supernumerary embryos, originating from a single IVF unit and not selected for use in treatment. Thus it was not possible to speculate how representative the findings would be of the better quality embryos transferred or frozen for each patient.

Wider implications of the findings: The data indicate that the BMI of women at conception is associated with distinct phenotypic changes in the embryo during the preimplantation period highlighting the importance of pre-pregnancy body weight in optimizing the chances of fertility and safeguarding maternal and offspring health. These changes to the metabolic fingerprint of human embryos are most likely a legacy of the ovarian conditions under which the oocyte has matured, may reduce the chances of conception for overweight women and provide good evidence that the metabolic profile of the early embryo is set by sub-optimal conditions around the time

of conception. The observed changes could indicate long-term implications for the health of the offspring of overweight and obese women

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Introduction

Rates of overweight and obesity are rising in women of reproductive age, in line with the global obesity epidemic. Overweight and obesity, defined as a body mass index within the ranges 25-29.9kg/m^2 and >30kg/m^2 respectively, are reported to have a negative impact on female reproductive health, in terms of reduced conception rates, increased rate of miscarriage (Boots and Stephenson, 2011), and maternal, fetal and neonatal complications (Balen, et al., 2007). In addition, being overweight during pregnancy increases the risk of developing gestational diabetes and large for gestational weight infants (Lawlor, et al., 2012); observations of particular importance given that weight at birth correlates with weight in later life (Rogers, et al., 2006). Epidemiological studies indicate that maternal body weight at conception and weight gain during the course of the pregnancy are associated with increased risk of cardiovascular and metabolic diseases in the offspring in later life (Lawlor, et al., 2012, Reynolds, et al., 2013). While it is widely accepted that many adult disorders have their origins in early development (Gluckman and Hanson, 2004) it is increasingly apparent that maternal nutrition in the periconceptual period can affect oocyte quality (Machtinger, et al., 2012), embryo development and offspring health (Connor, et al., 2012).

The ovarian follicle provides nutrients for the developing oocyte. For example, glucose present in the follicular cavity is principally converted to pyruvate by the granulosa-derived cumulus cells that surround the oocyte which is then transported into the oocyte where it is oxidised to provide ATP (Leese and Barton, 1984). In addition, mammalian oocytes contain a significant endogenous triglyceride repository, (Sturmey, et al., 2009) which provides a source of metabolic energy

during oocyte maturation (Dunning, et al., 2010, Ferguson and Leese, 2006, Sturmey and Leese, 2003).

The ovarian follicular environment is modified in obese women (Valckx, et al., 2012), with elevated levels of triglycerides, glucose and insulin (Robker, et al., 2009) the supply of which to the oocyte can have phenotypic consequences. For example, the exposure of bovine oocytes to a high-fat environment during final maturation reduces embryo viability post fertilisation, changes the expression of key metabolic genes and modifies metabolic activity in the resulting blastocysts (Van Hoeck, et al., 2011). While the pattern of metabolism in human oocytes and preimplantation embryos has been studied in some detail (Brison, et al., 2004, Butcher, et al., 1998, Gardner, et al., 2011, Gott, et al., 1990, Hardy, et al., 1989, Houghton, et al., 2002, Martin, et al., 1993, Sturmey, et al., 2009) little is known about whether the metabolic phenotype of the early embryo is sensitive to maternal body weight at the time of conception. This may be important, since the metabolic profile of preimplantation embryos is linked to ongoing viability (Brison, et al., 2004, Gardner, et al., 2011). Moreover, since critical epigenetic events occur during oogenesis (Kono, et al., 1996) and are completed postnatally (Lucifero, et al., 2004), a sub-optimal periconceptual environment may plausibly have a short- and/or long-term impact on development and set the early embryo on a metabolic trajectory that persists beyond the preimplantation period. This may increase the susceptibility of the offspring to the development of non-communicable diseases, including cancer, (Walker and Ho, 2012) cardiovascular disease and diabetes, the aetiology of which are considered to have a developmental component (Hanson and Gluckman, 2011).

121 The aim of this study was therefore to discover whether embryos derived from
122 oocytes of overweight and obese women display a compromised developmental and
123 metabolic profile. In order to carry out this work, we were fortunate to receive human
124 embryos conceived by In Vitro Fertilisation and donated for research purposes after
125 clinical treatment had been completed, with full, informed ethical consent.

Methods;

All research was carried out according to licence conditions of the Human Fertilisation and Embryology Authority (licence R0067), with full ethical approval (09/HI304/44).

Female and male BMI were recorded at the down-regulation appointment and at the commencement of treatment to ensure that the patients were weight-stable (defined as maintaining weight over a period of three months). Embryos that originated from patients classified as OW/OB (BMI ≥25kg/m^2) were compared to embryos derived from women of normal BMI (19 to 24.9kg/m^2). All patients indicating a willingness to be approached about research were given the opportunity to participate in the study. Only women with polycystic ovaries were excluded from the study as it was anticipated they might represent an additional subgroup with a specific metabolic profile linked with this condition.

Ovarian stimulation and oocyte collection were performed as described (Dickerson, et al., 2010). There were no differences in the stimulation regimens administered to patients in this study, however the duration and starting dose was adjusted according to patient age, AMH and antral follicle count. The oocyte retrieval was scheduled once the second largest follicle had reached 18mm and all follicles above 15mm were drained. Oocytes were cultured at 37^0C in 6% CO_2, 5% O_2, in Sage Quinn's Advantage (QA) Fertilisation Medium. Normally fertilised embryos were cultured until day 3 in QA Cleavage medium, and in QA Blastocyst medium until day 5 (all Sage QA products from Cooper Surgical, USA). Embryo transfer of one or two embryos was performed on day 3 or a single blastocyst on day 5, on the basis of the embryo

quality and surplus good quality blastocysts were cryopreserved for use in future treatment. Only then were patient consents checked and the remaining supernumerary developing embryos unsuitable for further clinical use donated to research, with full informed. Figure 1 depicts a summary of patients, oocytes and embryos included in each analysis. Observations were continued until day 9 or developmental arrest, to permit data capture from slower developing embryos which continued to show viability.

Oocyte assessments prior to ICSI and time-lapse development (observation and extended culture)

Oocyte diameters were measured during routine treatment and audited to assess differences in the quality of mature oocytes attained from normal weight and OW/OB women. Prior to ICSI two perpendicular measurements were taken of the ooplasm of 218 oocytes from 29 consecutive patients (see Figure 1A for details). As these measurements were taken prospectively, it was possible to track the onward developmental competence of the oocytes, based on their fertilisation, cleavage division to form embryos, development to form high scoring cleavage embryos, designated as having 6-8 cells on day 3 and a morphology score of grade 3 or above, and blastocyst formation on day 5.

Post transfer (68-116 hours post insemination) there was a total of 101 surplus embryos at various stages of continuing development which were placed into extended culture and observed using time-lapse technology (Primovision). Embryos were cultured in WOW dishes (Primovision, supplied by Vitrolife, Sweden), in culture conditions as described above. Recordings were made of specific developmental

timings/ events, using techniques described (Kirkegaard, et al., 2012). The time to reach 1) morula stage was defined as when all cells have fused, 2) unexpanded blastocyst; the first time a blastocyst is visible, 3) expanded blastocyst when the blastocoel expands 4) hatching; when the embryo escapes from the zona. The diameter of the blastocoel following collapse, recovery and hatching were also recorded.

Research embryo culture, assessment and metabolic assays

A second cohort of 29 consecutive patients presenting for IVF at the Hull IVF Unit donated a total of 150 embryos with full informed consent (See Figure 1C for details). Surplus embryos donated to research had their development stage recorded before being placed individually into 4µl drops of Earle's balanced salt solution, supplemented with 1mM glucose, 0.47mM pyruvate, 5mM Lactate, a physiological mixture of amino acids (Houghton, et al., 2002) (all obtained from Sigma-Aldrich Chemical, Poole, UK) and 0.5% (v/v) QA Serum Protein Substitute. Embryos were cultured under Sage Oil at 37^{o}C in 5% CO_2 for 24 hours, alongside embryo-free control drops. Embryos were subsequently moved to fresh culture droplets and developmental observations made. Those embryos that had failed to form a blastocyst, but continued to undergo cell divisions/ organisation were classified as cleavage stage (cell number) or morula, whereas those that reached the blastocyst stage were classified according to their degree of expansion (unexpanded, expanded, hatched). An embryo that failed to develop after 48 hours culture was considered arrested. Observations were ended on day 9. After incubation the spent culture medium was immediately frozen at -80^{0}C for later analysis.

Metabolic CORE profiles (Guerif et al., 2013) were determined by measuring the depletion and appearance of glucose, pyruvate, lactate and 18 amino acids, according to established techniques, that may be applied to individual oocytes and embryos:

(i) Glucose and pyruvate consumption and lactate production were measured using ultramicrofluorometric assays described by Leese and Barton (1984) and modified by Guerif et al., (2013). The assays are based on the enzymic phosphorylation of substrate and the subsequent consumption or generation of NADH or NADPH in coupled reactions which causes an increase in fluorescence which could be measured using a plate reader (Tecan Infinite M200) (excitation 340nm, fluorescence 459nm and above). All values are expressed as pmol embryo^{-1} hour^{-1}.

(ii) A coupled colorimetric assay was used to measure triglycerides as described by Sturmey and Leese (2003). Samples were pooled in groups of 2 to 5 embryos at equivalent development stages for each patient.

(iii) Spent culture droplets were analysed for amino acids using reverse-phase high performance liquid chromatography (HPLC), as described by Houghton et al., (2002). Average sums of amino acid production and depletion, were expressed in pmol embryo^{-1} hour^{-1} for day 5 to 9 of culture. All data were normalised to a non-metabolisable internal standard. Results were recorded according to stage reached at the end of the period of culture

Blastocyst cell counts

Expanded blastocysts (n=44- see Figure 1B for details) were fixed on day 7 of development using the differential staining technique based on that described by

Thouas et al., (2001) for mouse and bovine blastocysts. Chromatin-specific dyes were used to determine ICM and TE counts.

Statistical analysis

The data were compared between normal and OW/OB women and correlated retrospectively to the study end points: (a) blastocyst development and (b) clinical pregnancy outcome of the sibling embryos form transferred sibling embryos (which had not been analysed). Analyses were performed using SPSS, power calculations were performed based on the Birket and Day method (Birkett and Day, 1994), and studies were designed to achieve 80% power, unless otherwise stated. Leven's test for normality was performed and ANOVA with Tukey Kramer as indicated.

Univariate regression analysis was used to compare continuous data with paired t-tests to compare grouped two sample data. ANOVA was used to assess intra- and inter-patient variability within the embryo cohort in combination with multiple linear regression analysis to determine the predictive accuracy of metabolic profile on blastocyst development rate. To account for patient-specific effects in the triglyceride data, where samples where necessarily pooled into groups, Generalised Estimating Equations were used to separately model the mean response and within-cluster associations to reduce the variance and increase the power. Principal component analysis was used to reduce the dimensionality of the individual 18 amino acid measurements and adjust for multiple testing.

Results

In each of the experimental groups there were no significant differences in patient demographics; female age, AMH, male age and mean cycle number. However, follicle and oocyte numbers were significantly lower in the OW/OB groups compared to normal weight women in the observational study. In addition, male BMI was found to be elevated in partners of OW/OB women and has thus been controlled for appropriately, as described in the statistical methods. Similarly, intra-patient variability, which was evident for each cohort of oocytes / embryos has been taken in to consideration.

We first compared oocyte diameter from overweight and obese women (BMI >25kg/m^2) to women with a BMI <24.9kg/m^2 (n=29 women, 218 oocytes in total) since oocyte diameter has been proposed as a marker of oocyte developmental competence (Wickramasinghe, et al., 1991). There were three key observations; women with a higher BMI had smaller oocytes ($p<0.01$, Figure 2A) more likely to be in the lower quartile range for diameter; smaller oocytes were less likely to complete cleavage after fertilisation (Fig 2B, $r=0.23$, $p<0.001$), and to form blastocysts ($r=0.28$, $p<0.001$). Intriguingly, despite higher rates of cleavage-stage arrest, embryos from oocytes from OW/OB women that were capable of reaching the morula stage did so 17 hours earlier than counterparts from women with a BMI <25 (Figure 2C $p<0.001$). The resulting blastocysts from women with a BMI >25kg/m^2 at equivalent time points, tended to be smaller ($p=0.07$) at the point of maximum expansion, and had significantly lower cell counts (Fig 3A). In a multivariate analysis of the expanded blastocyst data, only female BMI was shown to be a significant predictor of cell count (Fig 3B), independent of embryo diameter, female age, cause of infertility and male

BMI. Furthermore, at equivalent time points, embryos from overweight and OW/OB mothers had fewer trophectoderm cells ($p<0.001$ (Fig 2B).

We next sought to discover whether the metabolic activity of 37 human blastocysts from 7 overweight/obese women differed from that of 113 blastocysts collected from 22 women who had a BMI <24.9. There were no other significant demographic differences between the groups including; age, cycle number and proportion IVF/ ICSI cycles as determined by independent sample t-test (Figure 1C), however male partners of OW/OB had significantly higher BMIs than those paired with normal weight women. We found that embryos from women with a BMI in excess of 25 kg/m^2 consumed significantly less glucose than embryos from women of a healthy weight at equivalent stages of development ($p<0.001$), whilst there were no significant changes in pyruvate uptake and lactate formation (Figure 4A). This pattern was consistent for each developmental stage. The reduced consumption of glucose occurred without a compensatory increase in pyruvate uptake, or of glycolytic activity as determined by lactate formation. In a multivariate analysis, developmental stage and female BMI were significant predictors of glucose uptake ($p<0.05$) and independent of male BMI, age, cause of infertility embryo grade and day each stage was attained. We were fortunate to identify a single male sperm donor that had been used to fertilise oocytes from 6 women, all of whom had a different BMI. With the male factor was controlled in this way, we were able to confirm the results of the multivariate analysis, which suggested that differences in embryo glucose consumption were independent of male BMI (Figure 4B);

Given these significant differences in glucose consumption, we compared the amino acid metabolism of embryos from overweight and obese women to those with a BMI <24.9 kg/m^2. Increased overall amino acid turnover is indicative of poor embryo

300 quality in terms of implantation potential (Brison, et al., 2004) and DNA damage
301 (Sturmey et al., 2009). Whilst we did not observe a significant difference in overall
302 amino acid turnover, we did find that embryos from overweight women had striking
303 differences in the consumption and release of individual amino acids compared to
304 those from healthy weight women. Thus, the appearances in the culture medium of
305 glutamate ($p<0.01$), aspartate ($p<0.001$), asparagine ($p<0.01$) and tryptophan
306 ($p<0.05$) were elevated while the depletion of serine ($p<0.01$) and glutamine ($p<0.01$)
307 were higher and that of isoleucine reduced in embryos from overweight group
308 compared to normal weight women. When the analysis was restricted to
309 developmental stage-matched blastocysts from the two BMI groupings the
310 differences were less pronounced, however embryos from overweight women still
311 depleted significantly more methionine than embryos from normal weight women
312 ($p<0.05$ Figure 4C).

313

314 Finally, we asked whether embryos from overweight women contained more
315 triglyceride than counterparts from women with a BMI <24.9kg/m^2. We observed that
316 day 9 blastocysts from women with a BMI >25kg/m^2 contained significantly more
317 triglyceride than comparable embryos from women with a BMI<24.9kg/m2 (Fig 5A;
318 $p<0.001$). Moreover embryos that arrested contained significantly more triglyceride
319 than those that completed development (11.32 ng vs 6.7 ng; $p<0.001$, Fig 5B). This
320 apparent retention of triglyceride and reduction in glucose consumption most likely
321 originates from the period of oocyte development, since all embryos were cultured in
322 equivalent conditions in vitro.

323

In terms of pregnancy outcome, the CORE glucose, lactate and pyruvate values given by sibling non-transferred embryos did not correlate with patient pregnancy outcome, however considerable intra-patient variability was observed. This variability was reduced when the analysis was limited to only developing sibling embryos alone, however no significant correlation with pregnancy was evident. Similarly, the mean turnover of amino acids for all embryos from women achieving pregnancies, despite appearing to be lower, was not significantly different to the non-pregnant group ($p=0.06$). When the analysis was limited to a comparison with developing blastocysts only, significant differences were observed in the production of asparagine ($p=0.02$) and glutamine ($p=0.04$), which were lower in the pregnant group, similarly the uptake of arginine ($p=0.03$) was lower. With regards to triglyceride content, this tended to be lower ($p=0.08$) in the sibling embryos from women achieving a pregnancy compared to those whose treatment was not successful.

Discussion

We report that embryos from overweight and obese women express a compromised developmental and metabolic phenotype. Specifically, oocytes from overweight and obese women are significantly smaller than those collected from women with a BMI considered to be in the healthy range. These smaller oocytes from overweight and obese women are less likely to reach the blastocyst stage, but those that do so, show accelerated preimplantation development and the subsequent blastocysts contain fewer cells, notably in the trophectoderm. These embryos also show significant metabolic abnormalities, with a diminished glucose consumption, altered profile of amino acid metabolism and strikingly, an increased endogenous triglyceride content. The data provide strong evidence for a direct link between maternal nutrition, the periconceptual environment, oocyte and preimplantation developmental competence and embryo metabolism, which could have long-term health implications for the offspring.

We found that oocytes collected from women with a BMI that exceeds 25 kg/m^2 are significantly smaller than comparable oocytes collected from women whose BMI is less than 25 kg/m^2; a finding in agreement with that of Marquard, et al., (2011). The impact of this observation is not yet clear, but Lucifero, et al., (2004) reported that the diameter of mouse oocytes was correlated with the accumulation of transcripts encoding for *Dnmt3a, Dnmt3b and Dnmt3L; e*nzymes which play a critical role in the establishment and maintenance of DNA methylation. Moreover, expression of one of these (DNMT3a) appears to be influenced by exposure of the oocyte to fatty acids (Van Hoeck, et al., 2011). This may indicate that the smaller oocytes from OW/OB women, exposed to elevated levels of fatty acids in the follicle, have dysregulated expression of enzymes with an essential role in regulating methylation and

365 epigenetic control in the resulting embryo Furthermore, fewer oocytes from
366 overweight and obese women were competent to reach the blastocyst once fertilised;
367 a finding that may contribute to the lower success rates of fertility treatment that have
368 been reported in overweight and obese women (Bellver, et al., 2010, Chavarro, et
369 al., 2012, Moragianni, et al., 2012, Shah, et al., 2011).

370

371 Although fewer embryos from overweight and obese women reached the blastocyst,
372 those that did so developed at a faster rate; an unexpected finding. Specifically,
373 embryos from overweight and obese women reached the morula stage of
374 development on average 17 hours faster than comparable embryos from women of a
375 healthy weight. This precocious pre-compaction development meant that blastocysts
376 were formed earlier in overweight and obese women, although the duration of
377 cavitation once the morula stage had been reached did not differ. The reasons
378 behind this precocious development are unclear, particularly given the recent report
379 by Bellver et al., (2013), who reported that embryos from overweight and obese
380 patients had similar timings in cell division to embryos from women of normal weight.
381 An important distinction between the work reported here and that of Bellver et al.,
382 (2013) relates the length of time that embryos were observed; Bellver et al., (2013)
383 reported findings for 72 hours post fertilisation, although they did conceded that
384 obesity may play an important role in the late stages of embryo development. We
385 now report for the first time that differences in developmental timing between
386 embryos from OW/OB patients only became apparent after 68 hours post
387 insemination.

388

We were surprised to find that the resulting blastocysts had fewer cells, notably in the trophectoderm lineage. The presence of fewer cells in the TE, from which the cytotrophoblast and syncitiotrophoblast will form, implies that at the time of implantation, there are fewer chorionic progenitor cells, which we propose may have an impact on the size and invasive properties of the trophoblast and subsequent placenta. Disrupted cell allocation may have downstream effects on placental growth, which is likely to be important since both low and high placental weight at birth have been shown in epidemiological studies to predict the likelihood of developing coronary heart disease, hypertension, stroke and cancer in adulthood (Barker, et al., 1990, Eriksson, et al., 2011).

In broad terms, the data on consumption of glucose by single human blastocysts are consistent with those previously reported (Gardner, et al., 2011, Hardy, et al., 1989). However, blastocysts from overweight and obese patients consumed significantly less glucose than equivalent embryos from women with a BMI <24.9 kg/m^2. A diminished capacity to metabolise glucose may be profound since there appears to be an evolutionarily conserved metabolic phenotype such that cleavage-stage embryos preferentially utilise pyruvate, while there is a characteristic increase in glucose consumption (Smith and Sturmey, 2013) during blastocyst formation. A reduction of glucose consumption at the blastocyst stage suggests some degree of metabolic remodelling in the blastocysts derived from oocytes collected form overweight and obese women. There are a number of reports that link embryo metabolism to ongoing developmental potential and Gardner et al., (2011) have proposed that low glucose consumption at the blastocyst stage relates to reduced human embryo viability. Given that in the current study, all of the embryos were cultured in equivalent conditions, we conclude that the origins of the altered glucose

metabolism in human blastocysts from overweight women can be traced back to conditions in the ovary. In addition, we were fortunate in having a cohort of 6 patients who received donor semen from a single donor, allowing us in essence to confirm the results from our statistical model which suggest that embryo metabolism is independent of male BMI. We observed a significant negative correlation between mean glucose consumption of blastocysts and female BMI, when the male contribution was controlled for, further supporting the conclusion that the origin of the metabolic alterations observed in the current study can be linked to the environment within the ovary. However, there is good evidence that male obesity can also impact on fertility and embryo viability (Bakos, et al., 2011). The molecular mechanism by which intra-follicular conditions modify the oocyte and subsequent embryo is unclear, but we consider it highly significant that bovine oocytes exposed to fatty acids at concentrations found in human ovarian follicles, (Robker, et al., 2009, Valckx, et al., 2012) display reduced glucose consumption in the subsequent blastocysts (Van Hoeck et al 2011) as in our present study.

The blastocysts of overweight and obese women consumed and produced a number of amino acids in increased quantities, compared to counterparts from women of a BMI <24.9 kg/m^2, further pointing to a degree of metabolic regulation. The increased appearance of aspartate and glutamate in embryos from OW/OB women might be indicative of a disrupted malate-aspartate shuttle (MAS), which plays a vital role in regulating glucose metabolism in mouse blastocysts (Mitchell, et al., 2009), and has a further function in regulating the REDOX status of the cytosol. It is also noteworthy that inadequate metabolism of amino acids leads to a delay in trophectoderm development through an mTOR-dependent pathway (Martin and Sutherland, 2001). Given that we observed a reduction in TE cells in the embryos from overweight and

441 obese patients, and reduced amino acid metabolism, it is tempting to speculate that
442 there is some degree of disruption to the mTOR signalling in these blastocysts. In
443 addition, embryos from overweight women consumed significantly more methionine
444 which plays an important role in the metabolic regulation of nucleotide synthesis and
445 methylation (Grillo and Colombatto, 2008); processes likely to be important up to the
446 stage of blastocyst expansion which coincides with the end of DNA demethylation
447 and loss of histone modifications and the onset of methylation (Feng, et al., 2010).

448

449 This is the first quantitative report of triglyceride in human blastocysts, the total
450 content of which was significantly lower than that observed in the domestic species
451 (Ferguson and Leese, 1999, Sturmey and Leese, 2003, Sturmey, et al., 2009). Total
452 endogenous triglyceride concentrations were lower in embryos that successfully
453 develop to the blastocyst stage and that blastocysts derived from oocytes of
454 overweight and obese patients contained significantly elevated levels of endogenous
455 triglyceride. It is unlikely that *de novo* synthesis of fatty acid occurs in the embryo,
456 although this cannot be discounted; more likely that oocytes present in the lipid-rich
457 follicles of overweight and obese women accumulate triglyceride from the
458 surrounding environment as reported by Aardema et al., (2011) and Ferguson and
459 Leese, (1999) for domestic species. This increased concentration of endogenous
460 triglyceride is further evidence of metabolic remodelling in blastocysts derived form
461 oocytes of overweight and obese women, and may explain the reduction in glucose
462 consumption, since it is widely established in somatic cells and tissues that an
463 increase in β-oxidation causes a reduction in glycolysis via elevated cytosolic citrate
464 levels which inhibit phosphofructokinase (Hue and Taegtmeyer, 2009).

465

The data comparing metabolic parameters to the pregnancy outcome of the sibling transferred embryos highlights the differences in developmental potential apparent in a cohort of embryos; a potential weakness of using the woman as the 'experimental unit' as opposed to individual embryos. This assumes that the intra follicular conditions were comparable in the ovaries of a patient. However in a given patient, even in follicles of comparable size, the degree of vascularization, oxygenation and level of nutrients have been shown to vary at the time of ovum retrieval (reviewed by Van Blerkom et al 2000). The more subtle differences in metabolic regulation and developmental competence of individual embryos could be attributed to these differences and further studies are required on the origin of intra follicular influences.

Studies on the consequences of maternal obesity have largely focussed on clinical complications for the mother during pregnancy and on offspring health; short- and long-term. Due to the complexities in working with human embryos and scarcity of material, much research on the impact of obesity on early development has been carried out in experimental animals (Van Hoeck, et al., 2011, Vogt, et al., 2014). Such data suggest that the early embryo is especially sensitive to nutritional and environmental challenges during the periconceptual period. Recent research efforts have begun to characterise the 're-programming' that occurs at this time, and the consequences for future development. We believe that the work presented here is the first to examine the impact of maternal overweight or obesity on the development and nutrition of human oocytes and preimplantation embryos and shows that maternal metabolic health acts via the ovary to alter the phenotype of the oocyte. These alterations persist in the zygote and manifest, in our study as a disrupted metabolism at the blastocyst stage with the potential to compromise fetal and offspring health.

Author roles: CL, HJL and RGS conceived the study. CL and RGS performed the research and analysed the data. CL, HJL and RGS wrote the manuscript. All authors had access to data at all times.

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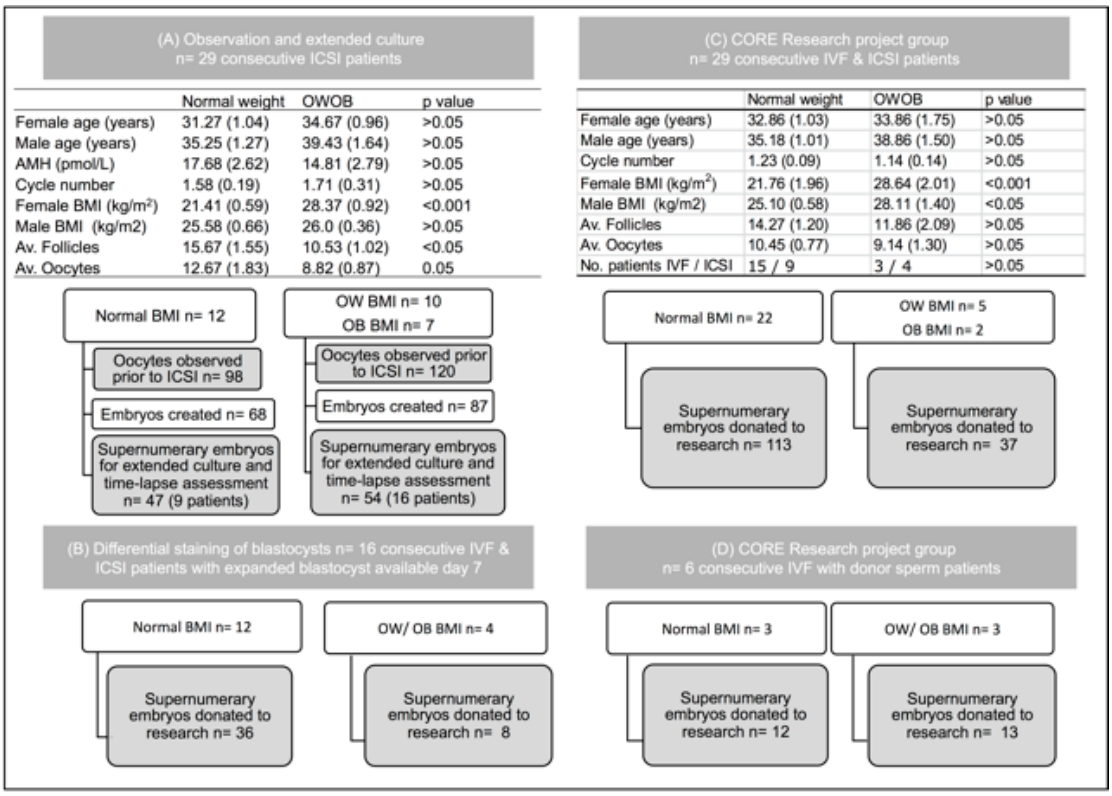
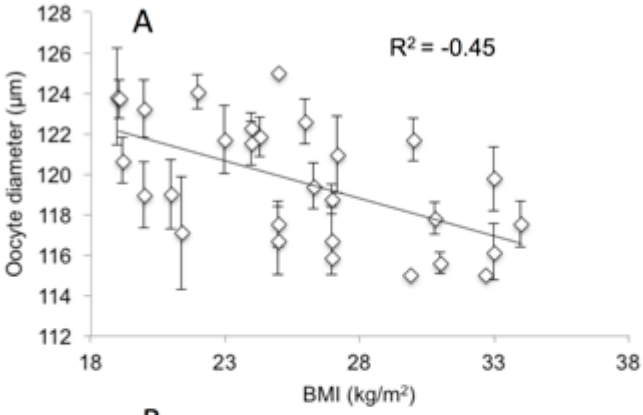
648

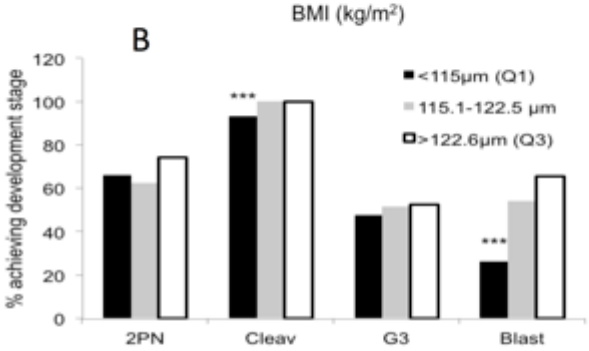
649 **Figure Legends**



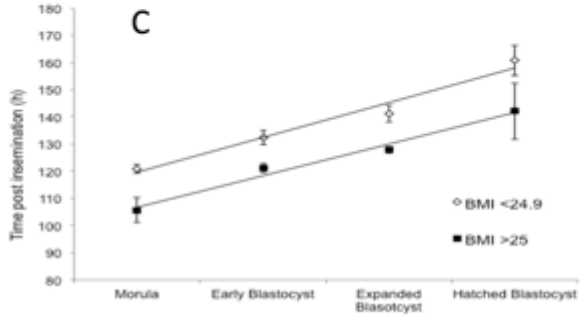
650 Figure 1 Schematic diagram of the study groups A-D

651 **Figure 1** Schematic diagram of the study groups A-D, indicating patient numbers and
652 demographics for each of the groupings (values are ± standard error). There were no
653 difference in patient age and cycle number, significant differences in follicle and oocyte
654 numbers in the observational study and male BMI in the CORE research grouping have been
655 appropriately controlled for in the multivariate statistical analyses. Shaded boxes indicate
656 oocytes and embryos included within the analysis.





Correlations (*r*)	Normal fertilisation (2PN)	Zygote cleavage (Cleav)	Day3 embryo quality (G3)	Day5 Blastocyst development (Blast)	BMI (kg/m²)
Oocyte diameter (µm)	0.13	0.23***	0.05	0.28***	-0.46***



657

658 **Figure 2** Developmental differences exist between oocytes generated from normal and
659 Overweight & Obese (OW/OB) women. **2A)** Oocyte diameter is inversely correlated to
660 female BMI. The data show mean (± SEM) oocyte diameters (n=218), recorded from 29
661 women (R^2 =-0.45; p=0.001). **2B)** The smallest oocytes, were significantly less likely to
662 cleave (p<0.001) and more likely to have originated from women with a higher BMI (p<0.001;
663 29 patients, n=155 embryos). **2C)** The time elapsed post insemination for morula stage to be
664 reached is shorter in embryos from OW/OB women compared to normal weight women
665 (p<0.001, 25 patients, n= 101 supernumerary embryos taken for extended culture

observation). As a consequence, post compaction stages of development arise earlier in embryos from OW/OB women, although the duration taken to complete blastocyst formation from the morula does not differ between OW/OB and normal weight women, suggesting precocious cleavage stage development. Note: discrepancies in numbers of embryos reflect exclusions from subsequent analysis due to fail-to fertilise oocytes (63 oocytes) and embryos transferred or cryopreserved as part of clinical treatment (54 embryos) – See Fig 1A for details.

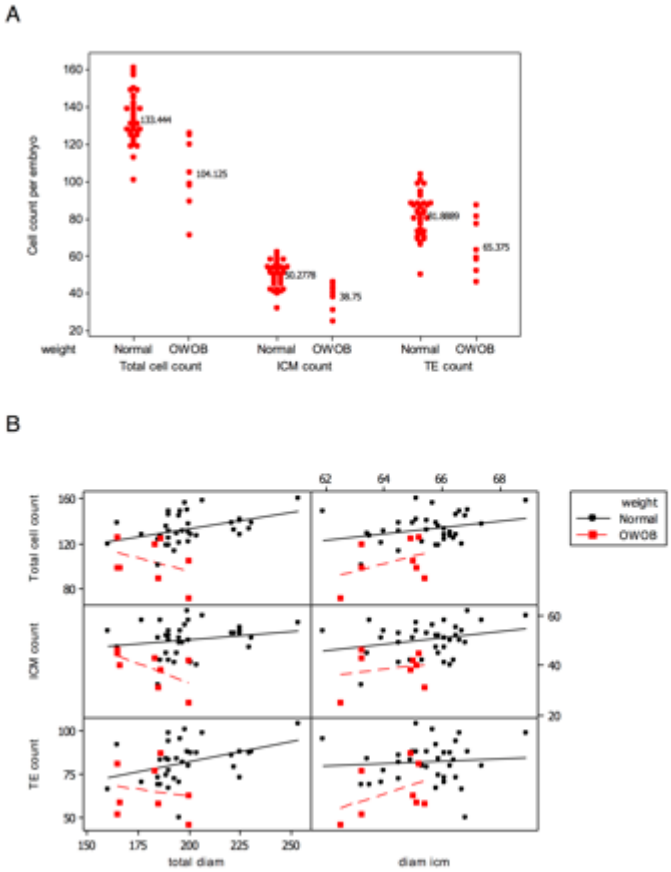


Figure 3; Total blastocyst cell counts, inner cell mass and trophectoderm cell counts for embryos that had been donated into research and had reached expanded blastocysts by day 7 of development (n= 44; see Fib 1B for details). 3**A)** Shows that total, ICM and TE cell counts were significantly lower in blastocysts from OW/OB compared to normal weight women (p=0.01; mean values displayed). **3B)** Shows the total, ICM and TE blastocyst cell counts, according to measures of total blastocyst diameter (µm) and ICM diameter (µm).

Blastocyst diameter shows a weak inverse relationship with female BMI (P=0.07). In a multivariate analysis diameter is not an independent predictor or cell count, whereas BMI is. The ICM count is predicted by female BMI and there is a trend for increased cell count with ICM diameter (p=0.08). The diameter of the ICM does not correlate with total cell count or total blastocyst diameter.

Figure 4 Significant differences in the metabolism of embryos generated from normal and Overweight & Obese (OW/OB) women that had been donated into research (see Fig 1C for details). **4A)** Embryos from women classified as OW/OB consume significantly less glucose than normal weight counterparts (p<0.001 37 embryos from 7 OW/OB women and 113 embryos from 22 normal weight women. Error bars represent 95% CI). **4B)** The glucose consumption of blastocysts is inversely correlated to female BMI (R^2=-0.61; p=0.001) and relates to maternal BMI with little paternal influence The oocytes in this figure were all fertilised by the same sperm donor, yet reduced glucose consumption was apparent in embryos from OW/OB women. (12 embryos from 3 women of a normal BMI and 13 embryos from 3 OW/OB women; See Fig 1D for details Data are expressed as mean ± SEM) **4C)** Amino acid depletion (negative values) and appearance (positive values) by blastocysts of equivalent stage for OW/OB (n=20 blastocysts from 37 embryos total) and normal weight women (n=27 blastocysts from 113 embryos; see Fig 1C for details). There are no significant differences in the sum of uptake and production, however blastocysts from OW/OB women depleted significantly more methionine than embryos from women of healthy weight (p=0.037). Error bars represent 95% CI.

Fig 4

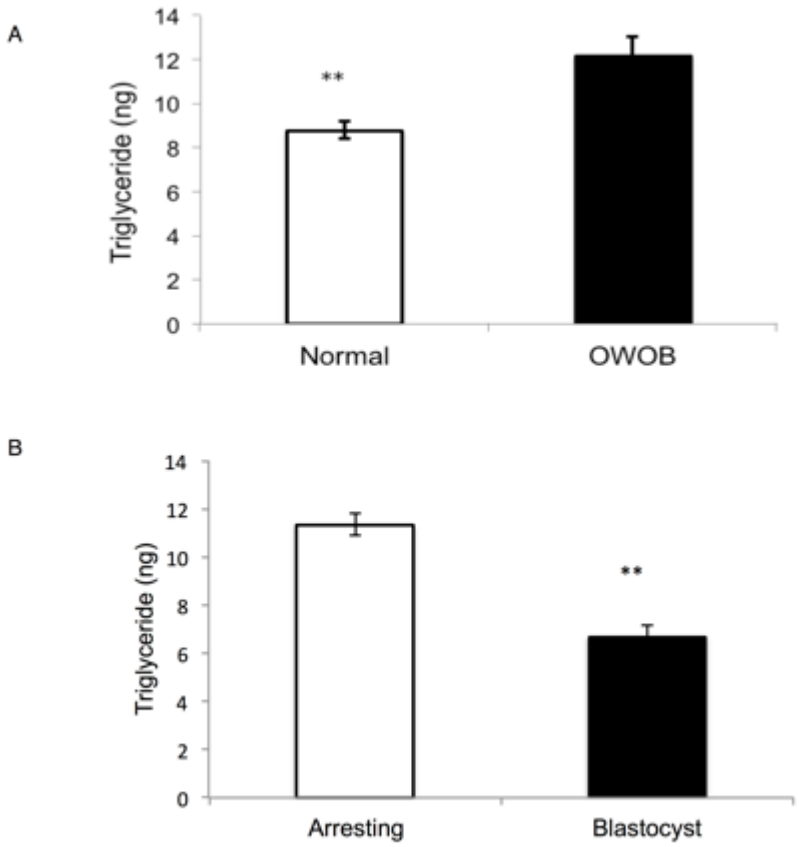


Figure 5: Triglyceride content of human embryos is influenced by maternal BMI. **5A)** Embryos that had been donated into research (see Fig 1C for details) derived from oocytes collected from OW/OB women contain significantly more triglyceride than those from healthy weight women (t=4.11, **p<0.001). **5B)** Embryos that arrest prior to the blastocyst stage (n=88) contain significantly more triglyceride than those capable of forming blastocysts (n=52; t=6.79, p<0.001), error bars represent standard error. Note, 10 embryos were unsuitable for analysis. This finding was consistent in both the normal weight and OW/OB women. Data are expressed as mean TG content ± SEM.