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

Christine Leary[1,2], Henry J. Leese[1], and Roger G. Sturmey[1,*]

[1]Centre for Cardiovascular and Metabolic Research, Hull York Medical School, University of Hull, Cottingham Road, Hull HU6 7RX, UK
[2]The Hull IVF Unit, The Women and Children's Hospital, Hull Royal Infirmary, Anlaby Road, Hull HU3 2JZ, UK

*Correspondence address. E-mail: sturmey@hyms.ac.uk

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STUDY QUESTION: Is the developmental timing and metabolic regulation disrupted in embryos from overweight or obese women?

SUMMARY ANSWER: Oocytes from overweight or obese women are smaller than those from women of healthy weight, yet post-fertilization they reach the morula stage faster and, as blastocysts, show reduced glucose consumption and elevated endogenous triglyceride levels.

WHAT IS KNOWN ALREADY: Female overweight and obesity 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 have 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 have related the data retrospectively to their body mass index (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 BMI in excess of 25 kg/m^2 to those from women of healthy weight. A Primovision Time-Lapse system was used to measure developmental kinetics and the non-invasive COnsumption/RElese of glucose, pyruvate, amino acids and lactate were measured on spent droplets of culture medium. Total triglyceride levels within individual embryos were also determined.

MAIN RESULTS AND THE ROLE OF CHANCE: Human oocytes from women presenting for fertility treatment with a BMI exceeding 25 kg/m^2 are smaller ($R^2 = -0.45$; $P = 0.001$) and therefore 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 blastocysts contain fewer cells notably in the trophectoderm ($P = 0.01$). The resulting blastocysts also 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, subanalysis 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 a high BMI of women at conception is associated with distinct phenotypic changes in the embryo during the preimplantation period, highlighting the importance of prepregnancy body weight in optimizing the chances of fertility and safeguarding maternal and offspring health. These changes to the metabolic fingerprint of human embryos which 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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Key words: obesity / human embryo metabolism / endogenous triglyceride / precocious development

Introduction

Rates of overweight and obesity (OW/OB) are rising in women of reproductive age, in line with the global obesity epidemic. OW/OB, defined as a BMI within the ranges 25–29.9 and >30 kg/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 and Anderson, 2007). In addition, being overweight during pregnancy increases the risk of developing gestational diabetes and large for gestational weight infants (Lawlor et al., 2012); these observations are 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 oxidized to provide ATP (Leese and Barton, 1984). In addition, mammalian oocytes contain a significant endogenous triglyceride repository (Sturmey et al., 2009a) that provides a source of metabolic energy during oocyte maturation (Sturmey and Leese, 2003; Ferguson and Leese, 2006; Dunning et al., 2010).

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-fertilization, 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 (Hardy et al., 1989; Gott et al., 1990; Martin et al., 1993; Butcher et al., 1998; Houghton et al., 2002; Brison et al., 2004; Sturmey et al., 2009b; Gardner et al., 2011), 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 post-natally (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).

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

Materials and Methods

All research was carried out according to licence conditions of the Human Fertilization and Embryology Authority (licence R0067), with full ethical approval (09/H1304/44).

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 3 months). Embryos that originated from patients classified as OW/OB (BMI ≥ 25 kg/m^2) were compared with embryos derived from women of normal BMI (19–24.9 kg/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 18 mm and all follicles above 15 mm were drained. Oocytes were cultured at 37°C in 6% CO_2, 5% O_2, in Sage Quinn's Advantage (QA) Fertilization Medium. Normally fertilized 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 of 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 were 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

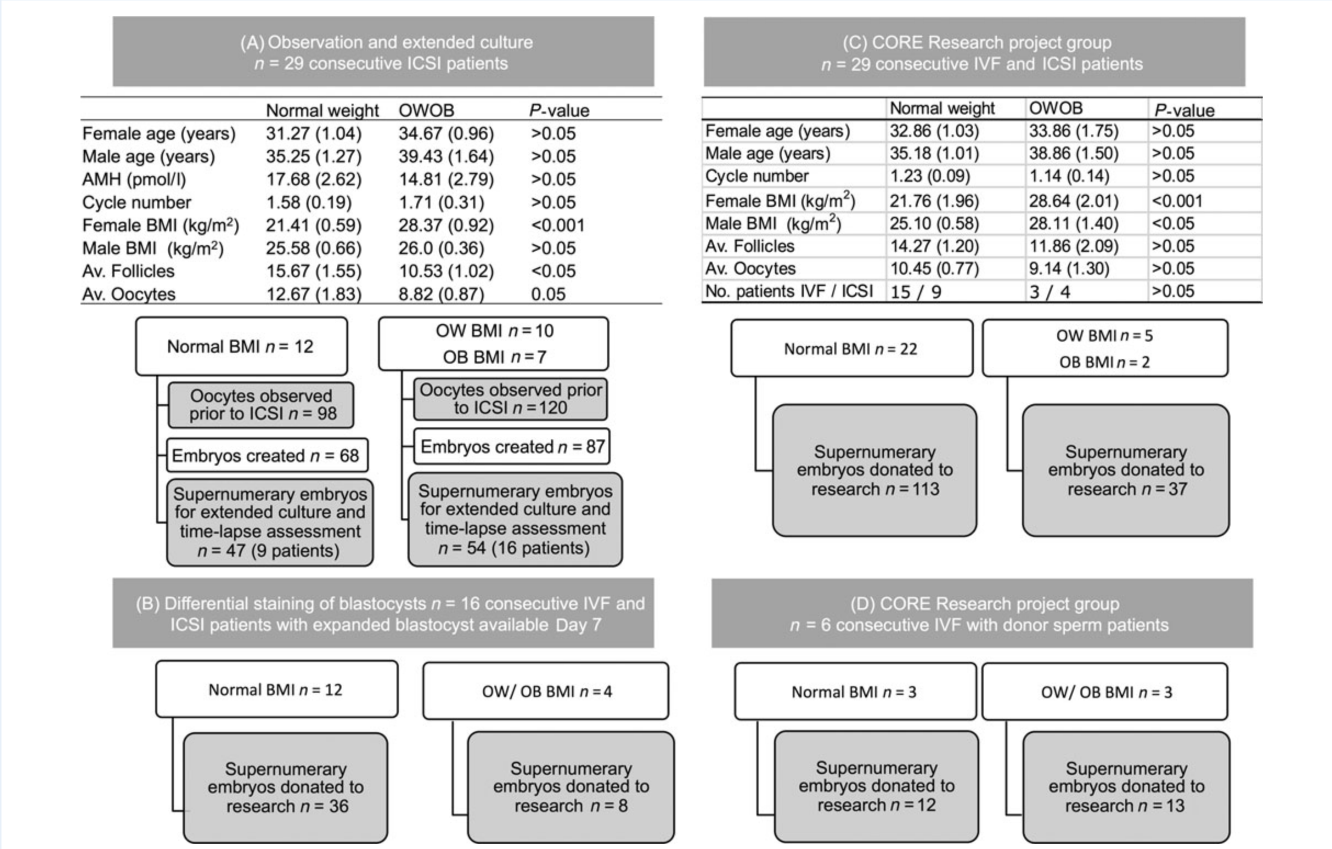


Figure 1 Schematic diagram of the study groups (**A**–**D**), indicating patient numbers and demographics for each of the groupings (values are ± standard error of the mean). There were no differences in patient age and cycle number, but significant differences in follicle and oocyte numbers and male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

were taken of the ooplasm of 218 oocytes from 29 consecutive patients (see Fig. 1A for details). As these measurements were taken prospectively, it was possible to track the onward developmental competence of the oocytes, based on their fertilization, 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 h 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 (i) morula stage was defined as when all cells have fused, (ii) unexpanded blastocyst was the first time a blastocyst is visible, (iii) expanded blastocyst was when the blastocoel expands and (iv) hatching was when the embryo escapes from the zona. The diameters 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 Fig. 1C for details). Surplus embryos donated to research had their development stage recorded before being placed individually into 4 µl drops of Earle's balanced salt solution, supplemented with 1 mM glucose, 0.47 mM pyruvate, 5 mM 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°C in 5% CO_2 for 24 h, 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/organization 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 and hatched). An embryo that failed to develop after 48 h in culture was considered arrested. Observations were ended on Day 9. After incubation, the spent culture medium was immediately frozen at −80°C for later analysis.

Metabolic COnsumption/RElese (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 340 nm, fluorescence 459 nm and above). All values are expressed as pmol embryo^{-1} h^{-1}.

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

(iii) Spent culture droplets were analysed for amino acids using reverse-phase high-performance liquid chromatography, as described by Houghton *et al.* (2002). Average sums of amino acid production and depletion were expressed in pmol embryo^{-1} h^{-1} for Days 5–9 of culture. All data were normalized to a non-metabolizable 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 Fig. 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: (i) blastocyst development and (ii) 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 and analysis of variance (ANOVA) with Tukey–Kramer were performed 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 necessarily pooled into groups, Generalized 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 with 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.

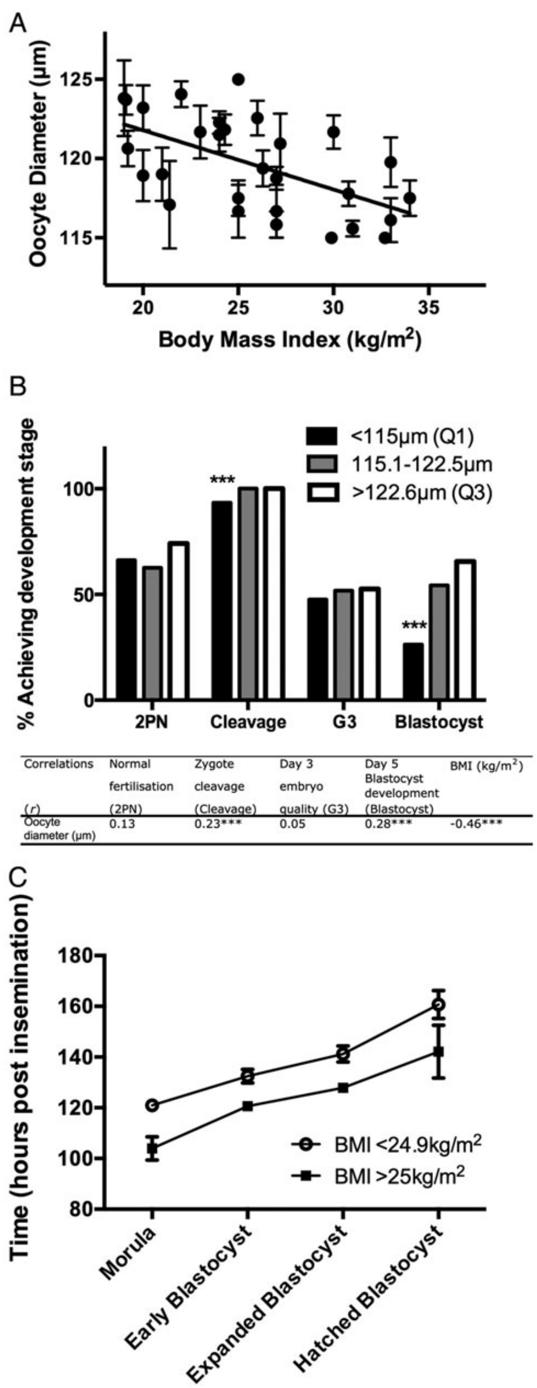
We first compared oocyte diameter from overweight and obese women (BMI > 25 kg/m^2) to women with a BMI < 24.9 kg/m^2 ($n = 29$ women, 218 oocytes in total) since oocyte diameter has been proposed as a marker of oocyte developmental competence (Wickramasinghe *et al.*, 1991). There were three key observations: women with a higher BMI had smaller oocytes ($P < 0.01$, Fig. 2A) more likely to be in the lower quartile range for diameter and smaller oocytes were less likely to complete cleavage after fertilization (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 h earlier than counterparts from women with a BMI < 25 (Fig. 2C $P < 0.001$). The resulting blastocysts from women with a BMI > 25 kg/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. 3B).

We next sought to discover whether the metabolic activity of 37 human blastocysts from 7 overweight and 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 (Fig. 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 (Fig. 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 fertilize oocytes from six 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 (Fig. 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 quality in terms of implantation potential (Brison *et al.*, 2004) and DNA damage (Sturmey *et al.*, 2009a). Whilst we did not observe a significant difference in overall amino acid turnover, we did find that embryos from overweight women had striking differences in the consumption and release of individual amino acids compared with those from healthy weight women. Thus, the appearances in the culture medium of glutamate ($P < 0.01$), aspartate ($P < 0.001$), asparagine ($P < 0.01$) and tryptophan ($P < 0.05$) were elevated while the depletion of serine ($P < 0.01$) and glutamine ($P < 0.01$) were higher and that of isoleucine reduced in embryos from overweight group compared with normal weight women. When the analysis was restricted to developmental stage-matched blastocysts from the two BMI groupings, the differences were less pronounced; however, embryos from overweight women still depleted significantly more methionine than embryos from normal weight women ($P < 0.05$ Fig. 4C).

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

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 with those whose treatment was not successful.



Correlations (*r*)	Normal fertilisation (2PN)	Zygote cleavage (Cleavage)	Day 3 embryo quality (G3)	Day 5 Blastocyst development (Blastocyst)	BMI (kg/m^2)
Oocyte diameter (µm)	0.13	0.23***	0.05	0.28***	-0.46***

Figure 2 Developmental differences exist between oocytes generated from normal and overweight and obese (OW/OB) women. (**A**) Oocyte diameter is inversely correlated to female BMI. The data show mean (±SEM) oocyte diameters ($n = 218$), recorded from 29 women ($R^2 = -0.45$; $P = 0.001$). (**B**) The smallest oocytes were significantly less likely to cleave ($P < 0.001$) and more likely to have originated from women with a higher BMI ($P < 0.001$; 29 patients, $n = 155$ embryos). (**C**) The time elapsed post-insemination for morula stage to be reached is shorter in embryos from OW/OB women compared with normal weight women ($P < 0.001$, 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 failed-to-fertilize oocytes (63 oocytes) and embryos transferred or cryopreserved as part of clinical treatment (54 embryos); see Figure 1A for details.

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

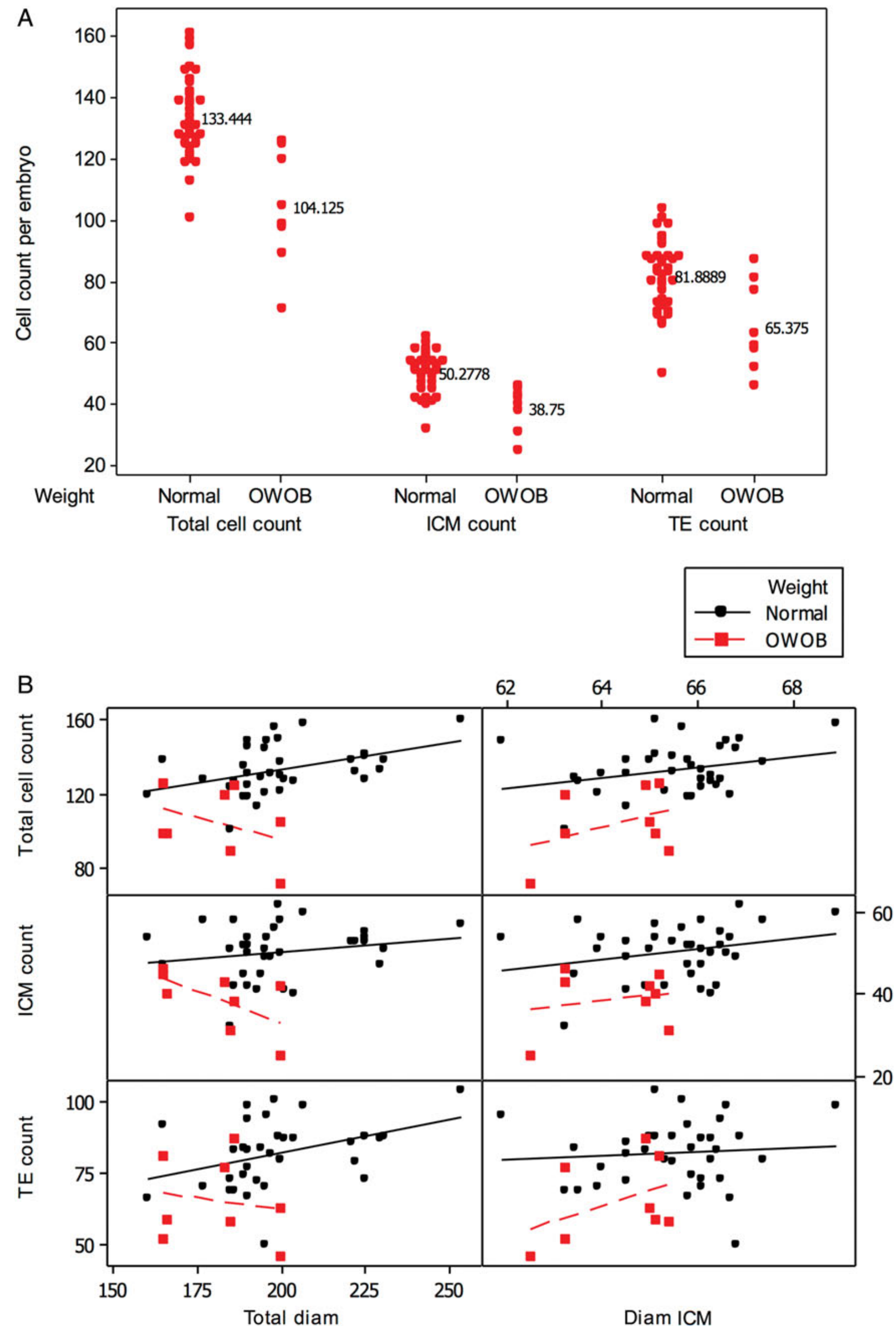


Figure 3 Total blastocyst cell counts, inner cell mass and trophectoderm cell counts for embryos that had been donated into research and had reached expanded blastocysts by Day 7 of development ($n = 44$; see Fig. 1B for details). (**A**) Shows that total, ICM and TE cell counts were significantly lower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

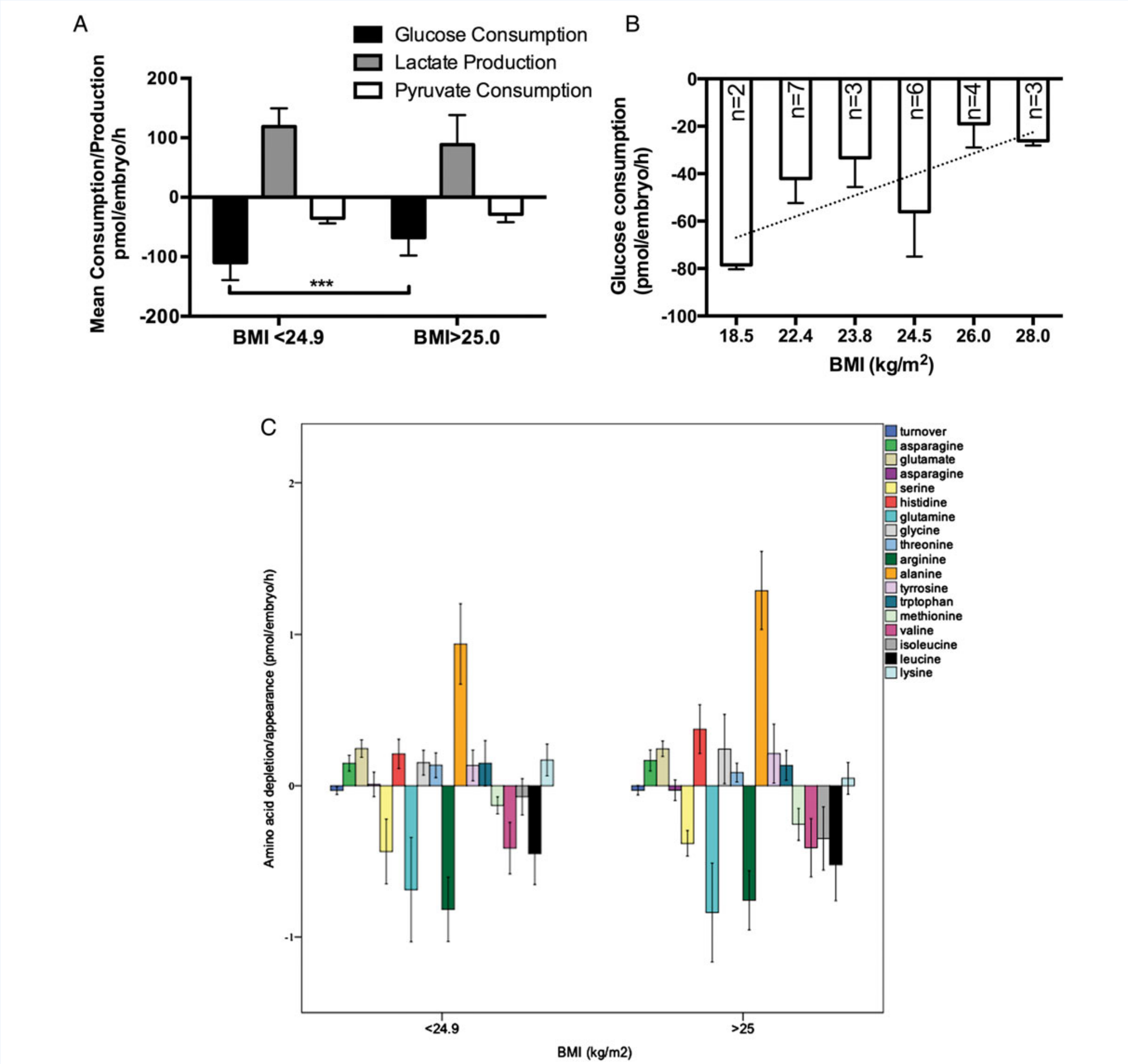


Figure 4 Significant differences in the metabolism of embryos generated from normal and OW/OB women that had been donated into research (see Fig. 1C for details). In all figures, negative bars represent substrate consumption, positive bars indicate appearance of a substrate. (**A**) Embryos from women 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). (**B**) 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 fertilized 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) (**C**) Amino acid depletion and appearance 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 were 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.

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 <25 kg/m^2; this 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

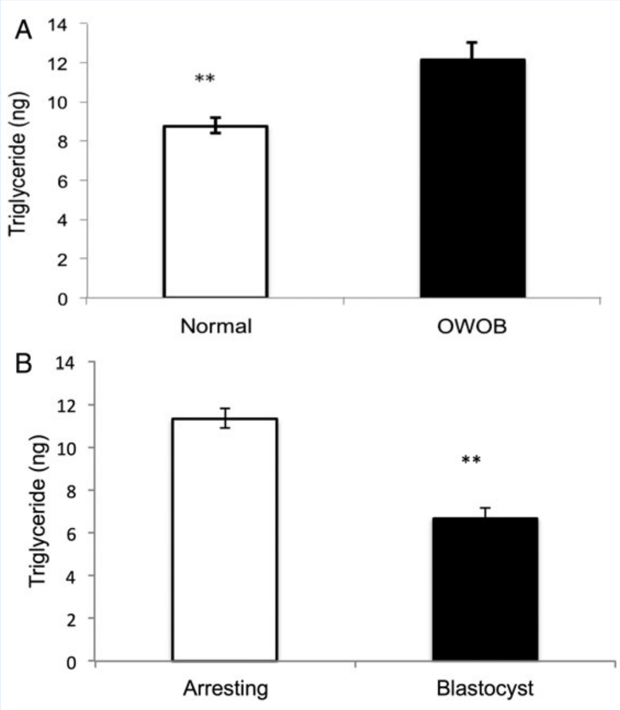


Figure 5 Triglyceride content of human embryos is influenced by maternal BMI. (**A**) Embryos that had been donated into research (see Fig. 1C for details) derived from oocytes collected from OW/OB women contain significantly more triglycerides than those from healthy weight women ($t = 4.11$, $^{**}P < 0.001$). (**B**) Embryos that arrested 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.

encoding for DNMT3a, DNMT3b and DNMT3L, enzymes 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 epigenetic control in the resulting embryo. Furthermore, fewer oocytes from overweight and obese women were competent to reach the blastocyst once fertilized; this finding that may contribute to the lower success rates of fertility treatment that have been reported in overweight and obese women (Bellver *et al.*, 2010; Shah *et al.*, 2011; Chavarro *et al.*, 2012; Moragianni *et al.*, 2012).

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

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 syncytiotrophoblast 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 (Hardy *et al.*, 1989; Gardner *et al.*, 2011). 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 metabolize glucose may be profound since there appears to be an evolutionarily conserved metabolic phenotype such that cleavage-stage embryos preferentially utilize 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 six 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 with 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, 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 mammalian target of rapamycin (mTOR)-dependent pathway (Martin and Sutherland, 2001). Given that we observed a reduction in TE cells in the embryos from overweight and obese patients, and reduced amino acid metabolism, it is tempting to speculate that there is some degree of disruption to the mTOR signalling in these blastocysts. In addition, embryos from overweight women consumed significantly more methionine which plays an important role in the metabolic regulation of nucleotide synthesis and methylation (Grillo and Colombatto, 2008); these processes are likely to be important up to the stage of blastocyst expansion which coincides with the end of DNA demethylation and loss of histone modifications and the onset of methylation (Feng *et al.*, 2010).

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

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; this is 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 , 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 focused on clinical complications for the mother during pregnancy and on offspring health, short- and long-term. Owing 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 characterize 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 as a disrupted metabolism at the blastocyst stage with the potential to compromise fetal and offspring health.

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Authors' roles

C.L., H.J.L. and R.G.S. conceived the study. C.L. and R.G.S. performed the research and analysed the data. C.L., H.J.L. and R.G.S. wrote the manuscript. All authors had access to the data at all times.

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Conflict of interest

There are no conflict of interests.

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