

1 **Human embryos from overweight and obese women display phenotypic and**
2 **metabolic abnormalities**

3 *Running title – Developmental disruption in embryos from overweight women*

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11 Key words: Obesity, Human Embryo Metabolism, Endogenous Triglyceride,
12 Precocious Development

13

14 **Abstract;**

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

17 **Summary answer:** Human oocytes from women presenting for fertility treatment
18 with a body mass index (BMI) exceeding 25kg/m² are smaller than those from
19 women of healthy weight, yet post fertilization they reach the morula stage faster.
20 Moreover, the blastocysts from overweight women show reduced glucose
21 consumption and contain elevated levels of endogenous triglyceride.

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

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

34 **Participants/materials, setting, methods:** In a clinical IVF setting, we compared
35 oocyte morphology and developmental kinetics of supernumerary embryos collected
36 from overweight and obese women, with a body mass index (BMI) in excess of
37 25kg/m² to those from women of healthy weight. Full informed consent was obtained.
38 A Primovision Time Lapse system was used to measure developmental kinetics and

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

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

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

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

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

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68 and the Hull York Medical School.

69 **Trial registration number: Not applicable.**

70

71 **Introduction**

72 Rates of overweight and obesity are rising in women of reproductive age, in line with
73 the global obesity epidemic. Overweight and obesity, defined as a body mass index
74 within the ranges 25-29.9kg/m² and >30kg/m² respectively, are reported to have a
75 negative impact on female reproductive health, in terms of reduced conception rates,
76 increased rate of miscarriage (Boots and Stephenson, 2011), and maternal, fetal and
77 neonatal complications (Balen, et al., 2007). In addition, being overweight during
78 pregnancy increases the risk of developing gestational diabetes and large for
79 gestational weight infants (Lawlor, et al., 2012); observations of particular importance
80 given that weight at birth correlates with weight in later life (Rogers, et al., 2006).
81 Epidemiological studies indicate that maternal body weight at conception and weight
82 gain during the course of the pregnancy are associated with increased risk of
83 cardiovascular and metabolic diseases in the offspring in later life (Lawlor, et al.,
84 2012, Reynolds, et al., 2013). While it is widely accepted that many adult disorders
85 have their origins in early development (Gluckman and Hanson, 2004) it is
86 increasingly apparent that maternal nutrition in the periconceptual period can affect
87 oocyte quality (Machtiger, et al., 2012), embryo development and offspring health
88 (Connor, et al., 2012).

89

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

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

98

99 The ovarian follicular environment is modified in obese women (Valckx, et al., 2012),
100 with elevated levels of triglycerides, glucose and insulin (Robker, et al., 2009) the
101 supply of which to the oocyte can have phenotypic consequences. For example, the
102 exposure of bovine oocytes to a high-fat environment during final maturation reduces
103 embryo viability post fertilisation, changes the expression of key metabolic genes and
104 modifies metabolic activity in the resulting blastocysts (Van Hoeck, et al., 2011).
105 While the pattern of metabolism in human oocytes and preimplantation embryos has
106 been studied in some detail (Brison, et al., 2004, Butcher, et al., 1998, Gardner, et
107 al., 2011, Gott, et al., 1990, Hardy, et al., 1989, Houghton, et al., 2002, Martin, et al.,
108 1993, Sturmey, et al., 2009) little is known about whether the metabolic phenotype of
109 the early embryo is sensitive to maternal body weight at the time of conception. This
110 may be important, since the metabolic profile of preimplantation embryos is linked to
111 ongoing viability (Brison, et al., 2004, Gardner, et al., 2011). Moreover, since critical
112 epigenetic events occur during oogenesis (Kono, et al., 1996) and are completed
113 postnatally (Lucifero, et al., 2004), a sub-optimal periconceptual environment may
114 plausibly have a short- and/or long-term impact on development and set the early
115 embryo on a metabolic trajectory that persists beyond the preimplantation period.
116 This may increase the susceptibility of the offspring to the development of non-
117 communicable diseases, including cancer, (Walker and Ho, 2012) cardiovascular
118 disease and diabetes, the aetiology of which are considered to have a developmental
119 component (Hanson and Gluckman, 2011).

120

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.

126 **Methods;**

127 All research was carried out according to licence conditions of the Human
128 Fertilisation and Embryology Authority (licence R0067), with full ethical approval
129 (09/HI304/44).

130

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

140

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

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

158

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

161 Oocyte diameters were measured during routine treatment and audited to assess
162 differences in the quality of mature oocytes attained from normal weight and OW/OB
163 women. Prior to ICSI two perpendicular measurements were taken of the ooplasm of
164 218 oocytes from 29 consecutive patients (see Figure 1A for details). As these
165 measurements were taken prospectively, it was possible to track the onward
166 developmental competence of the oocytes, based on their fertilisation, cleavage
167 division to form embryos, development to form high scoring cleavage embryos,
168 designated as having 6-8 cells on day 3 and a morphology score of grade 3 or
169 above, and blastocyst formation on day 5.

170

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

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

182

183 **Research embryo culture, assessment and metabolic assays**

184 A second cohort of 29 consecutive patients presenting for IVF at the Hull IVF Unit
185 donated a total of 150 embryos with full informed consent (See Figure 1C for details).
186 Surplus embryos donated to research had their development stage recorded before
187 being placed individually into 4µl drops of Earle's balanced salt solution,
188 supplemented with 1mM glucose, 0.47mM pyruvate, 5mM Lactate, a physiological
189 mixture of amino acids (Houghton, et al., 2002) (all obtained from Sigma-Aldrich
190 Chemical, Poole, UK) and 0.5% (v/v) QA Serum Protein Substitute. Embryos were
191 cultured under Sage Oil at 37°C in 5% CO₂ for 24 hours, alongside embryo-free
192 control drops. Embryos were subsequently moved to fresh culture droplets and
193 developmental observations made. Those embryos that had failed to form a
194 blastocyst, but continued to undergo cell divisions/ organisation were classified as
195 cleavage stage (cell number) or morula, whereas those that reached the blastocyst
196 stage were classified according to their degree of expansion (unexpanded,
197 expanded, hatched). An embryo that failed to develop after 48 hours culture was
198 considered arrested. Observations were ended on day 9. After incubation the spent
199 culture medium was immediately frozen at -80°C for later analysis.

200

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

205 (i) Glucose and pyruvate consumption and lactate production were
206 measured using ultramicrofluorometric assays described by Leese and
207 Barton (1984) and modified by Guerif et al., (2013). The assays are based
208 on the enzymic phosphorylation of substrate and the subsequent
209 consumption or generation of NADH or NADPH in coupled reactions
210 which causes an increase in fluorescence which could be measured using
211 a plate reader (Tecan Infinite M200) (excitation 340nm, fluorescence
212 459nm and above). All values are expressed as pmol embryo⁻¹ hour⁻¹.

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

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

223

224 **Blastocyst cell counts**

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

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

229

230 **Statistical analysis**

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

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

248

249 **Results**

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

258

259 We first compared oocyte diameter from overweight and obese women (BMI
260 $>25\text{kg/m}^2$) to women with a BMI $<24.9\text{kg/m}^2$ ($n=29$ women, 218 oocytes in total)
261 since oocyte diameter has been proposed as a marker of oocyte developmental
262 competence (Wickramasinghe, et al., 1991). There were three key observations;
263 women with a higher BMI had smaller oocytes ($p<0.01$, Figure 2A) more likely to be
264 in the lower quartile range for diameter; smaller oocytes were less likely to complete
265 cleavage after fertilisation (Fig 2B, $r=0.23$, $p<0.001$), and to form blastocysts ($r=0.28$,
266 $p<0.001$). Intriguingly, despite higher rates of cleavage-stage arrest, embryos from
267 oocytes from OW/OB women that were capable of reaching the morula stage did so
268 17 hours earlier than counterparts from women with a BMI <25 (Figure 2C $p<0.001$).
269 The resulting blastocysts from women with a BMI $>25\text{kg/m}^2$ at equivalent time points,
270 tended to be smaller ($p=0.07$) at the point of maximum expansion, and had
271 significantly lower cell counts (Fig 3A). In a multivariate analysis of the expanded
272 blastocyst data, only female BMI was shown to be a significant predictor of cell count
273 (Fig 3B), independent of embryo diameter, female age, cause of infertility and male

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

276

277 We next sought to discover whether the metabolic activity of 37 human blastocysts
278 from 7 overweight/obese women differed from that of 113 blastocysts collected from
279 22 women who had a BMI < 24.9 . There were no other significant demographic
280 differences between the groups including; age, cycle number and proportion IVF/
281 ICSI cycles as determined by independent sample t-test (Figure 1C), however male
282 partners of OW/OB had significantly higher BMIs than those paired with normal
283 weight women. We found that embryos from women with a BMI in excess of 25
284 kg/m^2 consumed significantly less glucose than embryos from women of a healthy
285 weight at equivalent stages of development ($p < 0.001$), whilst there were no
286 significant changes in pyruvate uptake and lactate formation (Figure 4A). This pattern
287 was consistent for each developmental stage. The reduced consumption of glucose
288 occurred without a compensatory increase in pyruvate uptake, or of glycolytic activity
289 as determined by lactate formation. In a multivariate analysis, developmental stage
290 and female BMI were significant predictors of glucose uptake ($p < 0.05$) and
291 independent of male BMI, age, cause of infertility embryo grade and day each stage
292 was attained. We were fortunate to identify a single male sperm donor that had been
293 used to fertilise oocytes from 6 women, all of whom had a different BMI. With the
294 male factor was controlled in this way, we were able to confirm the results of the
295 multivariate analysis, which suggested that differences in embryo glucose
296 consumption were independent of male BMI (Figure 4B);

297 Given these significant differences in glucose consumption, we compared the amino
298 acid metabolism of embryos from overweight and obese women to those with a BMI
299 $< 24.9 \text{ kg/m}^2$. 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 (Sturmeijer 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 $<24.9\text{kg/m}^2$. We observed that
316 day 9 blastocysts from women with a BMI $>25\text{kg/m}^2$ contained significantly more
317 triglyceride than comparable embryos from women with a BMI $<24.9\text{kg/m}^2$ (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

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

337

338

339 Discussion

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

353

354 We found that oocytes collected from women with a BMI that exceeds 25 kg/m² are
355 significantly smaller than comparable oocytes collected from women whose BMI is
356 less than 25 kg/m²; a finding in agreement with that of Marquard, et al., (2011). The
357 impact of this observation is not yet clear, but Lucifero, et al., (2004) reported that the
358 diameter of mouse oocytes was correlated with the accumulation of transcripts
359 encoding for *Dnmt3a*, *Dnmt3b* and *Dnmt3L*; enzymes which play a critical role in the
360 establishment and maintenance of DNA methylation. Moreover, expression of one of
361 these (DNMT3a) appears to be influenced by exposure of the oocyte to fatty acids
362 (Van Hoeck, et al., 2011). This may indicate that the smaller oocytes from OW/OB
363 women, exposed to elevated levels of fatty acids in the follicle, have dysregulated
364 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

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

399

400 In broad terms, the data on consumption of glucose by single human blastocysts are
401 consistent with those previously reported (Gardner, et al., 2011, Hardy, et al., 1989).
402 However, blastocysts from overweight and obese patients consumed significantly
403 less glucose than equivalent embryos from women with a BMI <24.9 kg/m². A
404 diminished capacity to metabolise glucose may be profound since there appears to
405 be an evolutionarily conserved metabolic phenotype such that cleavage-stage
406 embryos preferentially utilise pyruvate, while there is a characteristic increase in
407 glucose consumption (Smith and Sturmey, 2013) during blastocyst formation. A
408 reduction of glucose consumption at the blastocyst stage suggests some degree of
409 metabolic remodelling in the blastocysts derived from oocytes collected from
410 overweight and obese women. There are a number of reports that link embryo
411 metabolism to ongoing developmental potential and Gardner et al., (2011) have
412 proposed that low glucose consumption at the blastocyst stage relates to reduced
413 human embryo viability. Given that in the current study, all of the embryos were
414 cultured in equivalent conditions, we conclude that the origins of the altered glucose

415 metabolism in human blastocysts from overweight women can be traced back to
416 conditions in the ovary. In addition, we were fortunate in having a cohort of 6
417 patients who received donor semen from a single donor, allowing us in essence to
418 confirm the results from our statistical model which suggest that embryo metabolism
419 is independent of male BMI. We observed a significant negative correlation between
420 mean glucose consumption of blastocysts and female BMI, when the male
421 contribution was controlled for, further supporting the conclusion that the origin of the
422 metabolic alterations observed in the current study can be linked to the environment
423 within the ovary. However, there is good evidence that male obesity can also impact
424 on fertility and embryo viability (Bakos, et al., 2011). The molecular mechanism by
425 which intra-follicular conditions modify the oocyte and subsequent embryo is unclear,
426 but we consider it highly significant that bovine oocytes exposed to fatty acids at
427 concentrations found in human ovarian follicles, (Robker, et al., 2009, Valckx, et al.,
428 2012) display reduced glucose consumption in the subsequent blastocysts (Van
429 Hoeck et al 2011) as in our present study.

430

431 The blastocysts of overweight and obese women consumed and produced a number
432 of amino acids in increased quantities, compared to counterparts from women of a
433 BMI <24.9 kg/m², further pointing to a degree of metabolic regulation. The increased
434 appearance of aspartate and glutamate in embryos from OW/OB women might be
435 indicative of a disrupted malate-aspartate shuttle (MAS), which plays a vital role in
436 regulating glucose metabolism in mouse blastocysts (Mitchell, et al., 2009), and has
437 a further function in regulating the REDOX status of the cytosol. It is also noteworthy
438 that inadequate metabolism of amino acids leads to a delay in trophectoderm
439 development through an mTOR-dependent pathway (Martin and Sutherland, 2001).
440 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 from
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

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

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

491

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493 and analysed the data. CL, HJL and RGS wrote the manuscript. All authors had access to
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501

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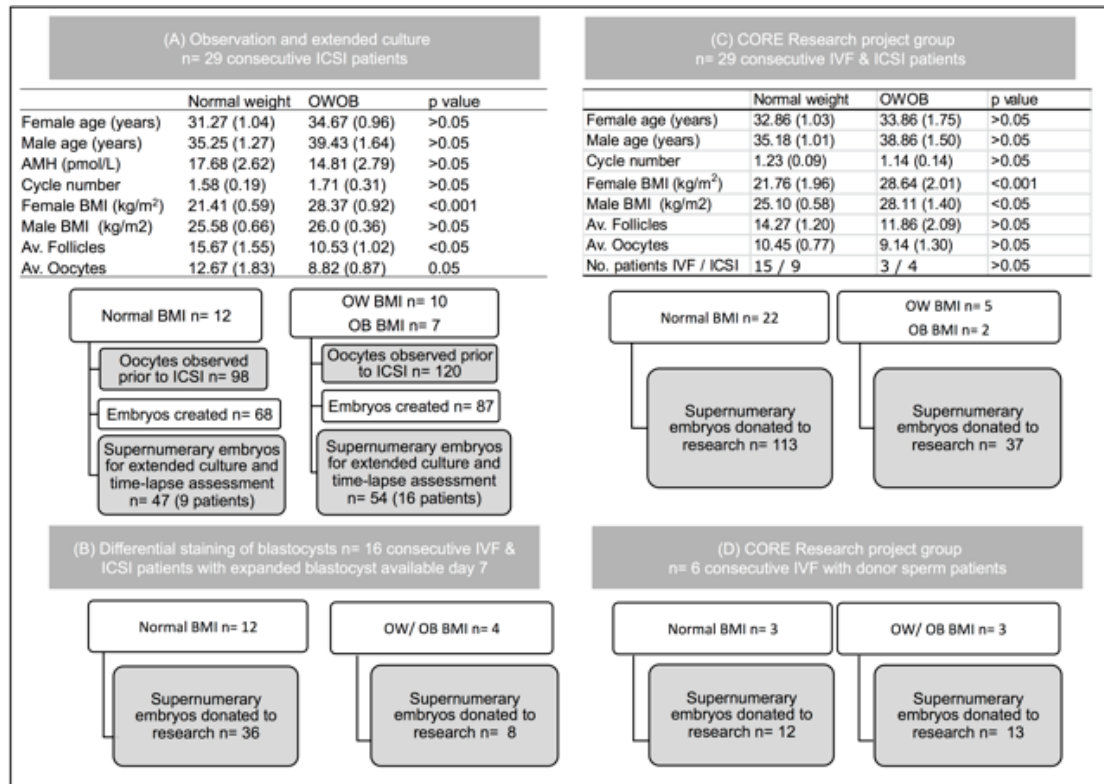
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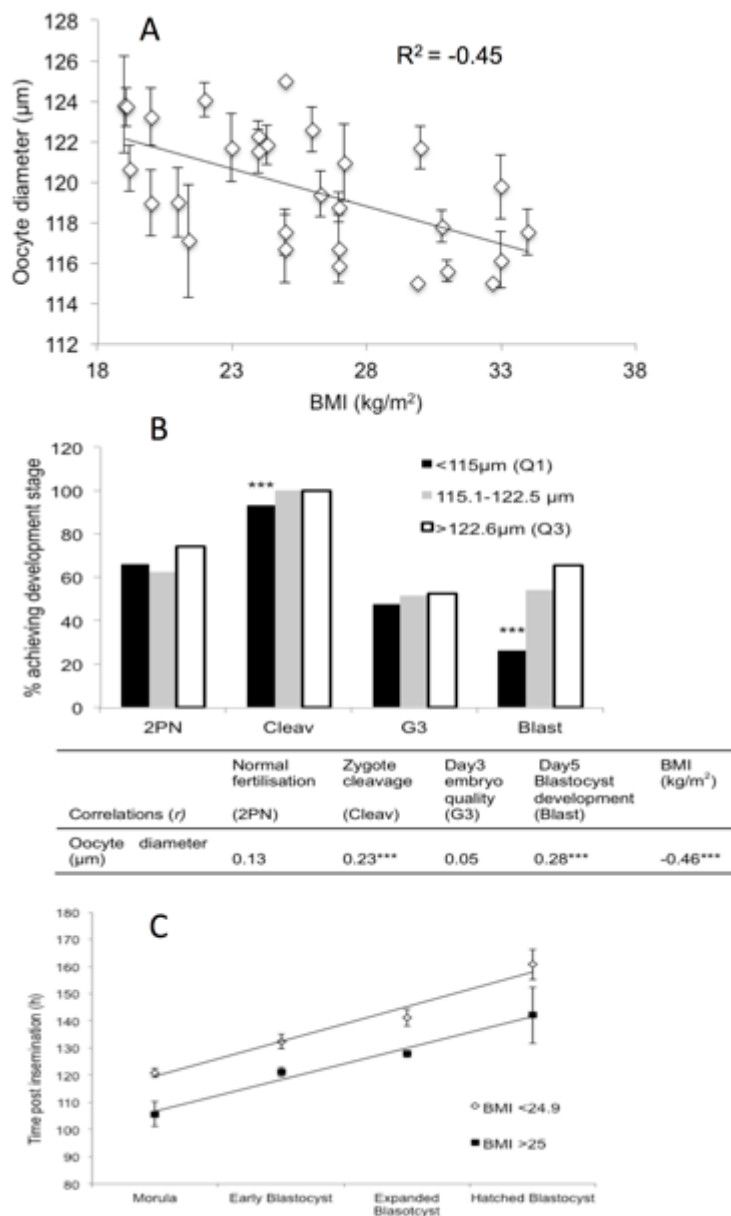
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648

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

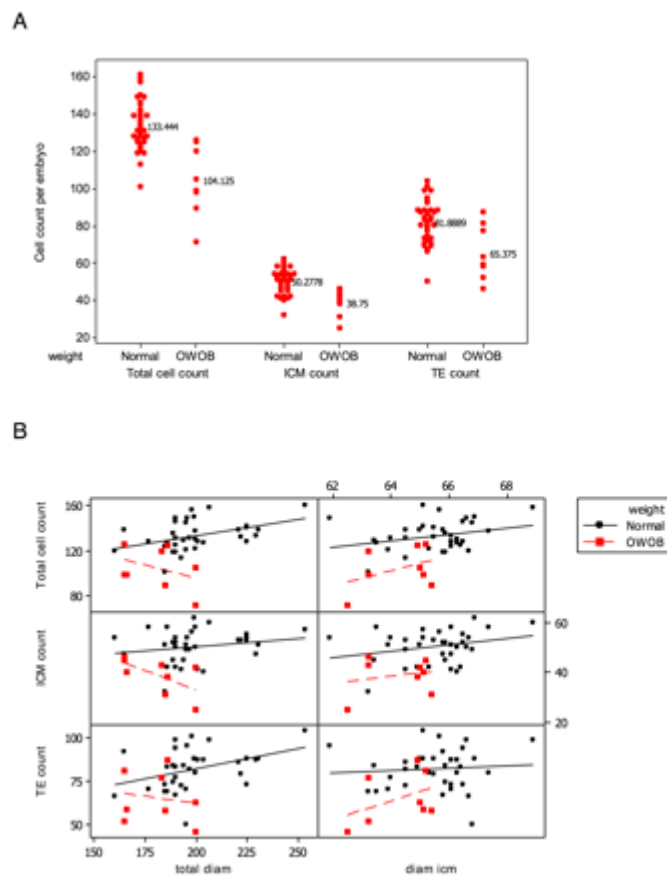
651 **Figure 1** Schematic diagram of the study groups A-D, indicating patient numbers and
 652 demographics for each of the groupings (values are \pm standard error). There were no
 653 difference in patient age and cycle number, significant differences in follicle and oocyte
 654 numbers in the observational 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.



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 (\pm 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

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



673

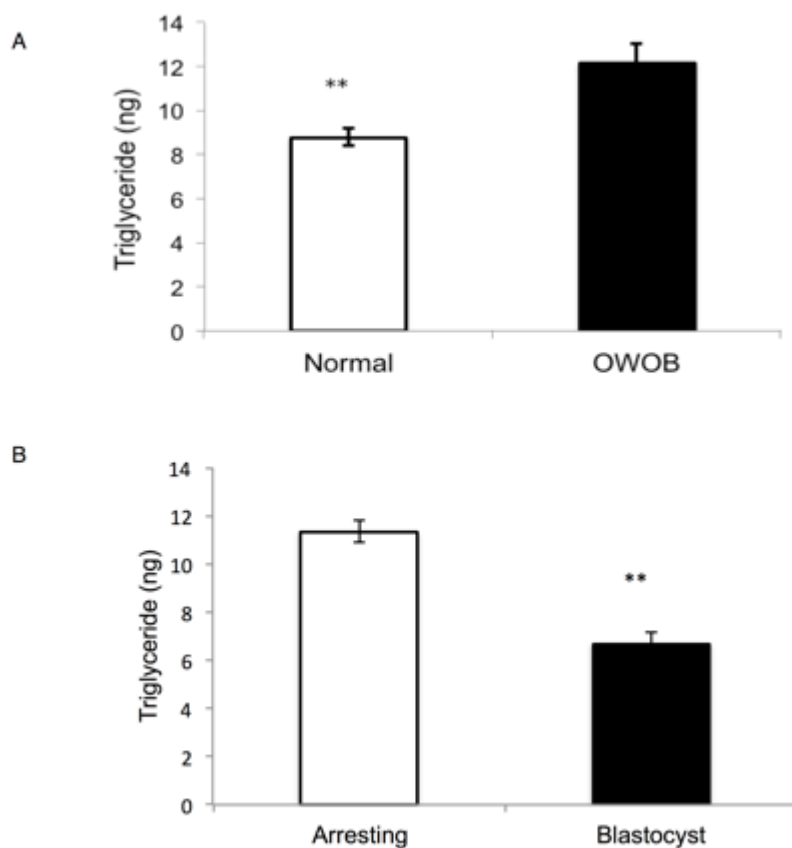
674 **Figure 3;** Total blastocyst cell counts, inner cell mass and trophoctoderm cell counts for
 675 embryos that had been donated into research and had reached expanded blastocysts by day
 676 7 of development (n= 44; see Fib 1B for details). **3A)** Shows that total, ICM and TE cell
 677 counts were significantly lower in blastocysts from OW/OB compared to normal weight
 678 women ($p=0.01$; mean values displayed). **3B)** Shows the total, ICM and TE blastocyst cell
 679 counts, according to measures of total blastocyst diameter (μm) and ICM diameter (μm).

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

685

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

Fig 4



702

703 **Figure 5:** Triglyceride content of human embryos is influenced by maternal BMI. **5A)**

704 Embryos that had been donated into research (see Fig 1C for details) derived from oocytes

705 collected from OW/OB women contain significantly more triglyceride than those from healthy

706 weight women ($t=4.11$, $**p<0.001$). **5B)** Embryos that arrest prior to the blastocyst stage707 ($n=88$) contain significantly more triglyceride than those capable of forming blastocysts ($n=52$;708 $t=6.79$, $p<0.001$), error bars represent standard error. Note, 10 embryos were unsuitable for

709 analysis. This finding was consistent in both the normal weight and OW/OB women. Data are

710 expressed as mean TG content \pm SEM.