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Increased miscarriage of euploid pregnancies in obese women undergoing cryopreserved embryo transfer

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KEY MESSAGE

Women who are overweight or obese have a significantly higher rate of miscarriage of genetically normal pregnancies when compared with lean women. This difference in miscarriage risk still remains significant even when accounting for relevant confounders such as maternal age, obstetric history and cause of infertility.

A B S T R A C T

Obesity is known to be associated with an increased risk of miscarriage after natural and assisted conception. Although most sporadic miscarriages are caused by genetic abnormalities, it is presently uncertain if genetics is also the underlying mechanism leading to increased pregnancy loss seen in obese women. Karyotyping of the products of conception suggests a reduced rate of fetal aneuploidy in miscarriages from obese compared with lean individuals. Karyotype analysis, however, is prone to false negative results because of inadvertent culture of maternal rather than fetal tissue. Therefore, to better analyse the effect of the genetic status on obesity-related miscarriage, we retrospectively analysed the outcomes 125 consecutive cryopreserved embryo transfer cycles resulting in a pregnancy after screening for genetic normality using comparative genomic hydridization. Lean individuals (body mass index 18.5–24.9 kg/m^2) had a significantly lower rate of miscarriages (14.2%) than overweight (29.1%) or obese (41.9%) women (P = 0.001); this relationship remained significant (P = 0.023) even after adjusting for relevant confounders, e.g. maternal age, cause of infertility, number of previous IVF cycles, type of frozen embryo transfer cycle or past obstetric history. These results support a non-genetic cause for obesity-related miscarriage.

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http://dx.doi.org/10.1016/j.rbmo.2016.09.011

Introduction

The prevalence of obesity has risen significantly in the past 3 decades, with data now suggesting that most adults in the developed world are either overweight or obese (Flegal et al., 2012). Although it is already recognised that obesity has a major effect on general heath, e.g. diabetes, hypertension and cardiovascular disease, increasing evidence shows that female obesity has a negative effect on reproductive health, such as increased time to natural conception (Hassan and Killick, 2004) and an increased rate of miscarriage (Metwally et al., 2008).

It is presently uncertain whether the increased risk of miscarriage with obesity is related to problems with the embryo caused by impaired oocyte quality, impaired uterine function or a combination of the two. Up to 70% of sporadic miscarriages are known to be associated with lethal numerical chromosomal errors, i.e. trisomy, monosomy and polyploidy (Sugiura-Ogasawara, 2015); however, it is questionable whether embryo genetic status is primarily responsible for increased miscarriage risk among obese women. Preimplantation genetic screening (PGS) of embryos during IVF treatment has failed to show any increase in the rate of embryonic aneuploidy with increasing maternal body mass index (BMI) (Goldman et al., 2015). Furthermore, several previous studies have reported that aneuploid miscarriage is actually less common in obese women than lean women (Boots et al., 2014; Kroon et al., 2011; Landres et al., 2010), thereby suggesting a non-genetic mechanism for pregnancy loss. As these miscarriage studies relied on karyotyping of the products of conception, however, a diagnostic test known to overestimate the rate of true embryonic euploidy owing to inadvertent culture of maternal cells (Boots et al., 2014), there is still some uncertainty about the possible role that genetic abnormality plays in obesity-related miscarriages.

Preimplantation genetic screening of embryos using techniques such as comparative genomic hydridization (CGH) offers significant advantages over traditional karyotyping of the products of conception (POC) when ascertaining a potential genetic cause for pregnancy failure in obese women. First, PGS involves the direct biopsy of the embryo with no potential for contamination of the biopsy with maternal cells, thereby increasing the diagnostic accuracy. Previous studies combining traditional karyotyping of POC with microsatellite analysis has reported that as many as 88% of karyotypes determined euploid XX miscarriages are actually caused by maternal cell contamination, a significant false negative result (Boots et al., 2014). Second, array-based CGH testing of embryos examines the genetic normality of the conceptus with a higher resolution (1 Mb DNA using the BlueGnome platform) than can occur with traditional G banding karyotyping of POC (resolution in excess of 10 Mb DNA) (Shaffer and Bejjani, 2004). As a result, CGH testing of embryos has the potential of identifying gains or losses of sub-microscopic amounts of DNA across the whole embryo genome that would be missed in G band karyotyping of miscarriage products of conception (Bagheri et al., 2015). A recent meta-analysis of nine studies comparing chromosomal microarray-based analysis with traditional karyotyping of POC concluded that array technology had the ability to detect an additional 13% of chromosomal abnormalities over conventional karyotyping (Dhillon et al., 2014).

Given this background, we hypothesise that the increased rate of pregnancy loss seen in obese women is likely to be related to an aberrant uterine implantation process rather than genetic abnormality in the embryo. To test this hypothesis, we elected to retrospectively analyse pregnancy outcomes in women who become pregnant after the transfer of a confirmed euploid embryo in a cryopreserved IVF cycle and then correlate those pregnancy outcomes with maternal BMI.

Materials and methods

Study population

Patients undertaking PGS treatment at a private infertility unit between November 2012 and December 2014 were included in the study. Indications for PGS included advanced maternal age, previous IVF treatment failure, and a wish to improve the efficiency of subsequent frozen embryo transfer (FET) cycles. Routine IVF treatment protocols using a GnRH antagonist regimen were used as previously reported (Tremellen and Lane, 2010), with genetic testing of embryos occurring only on those embryos destined for cryopreservation, not fresh transfer. All participants underwent a subsequent cryopreserved transfer of a single euploid embryo at least one menstrual cycle after their stimulated IVF cycle. Patients using donated oocytes or surrogacy were excluded from the analysis, and each patient is only represented once within the study cohort (their first transfer of a known euploid embryo which resulted in a pregnancy confirmed on serum beta-HCG assessment at 4 weeks gestation).

Before starting the cycle, BMI was calculated using the formula weight/height^2. All measurements were made by clinic staff within 3 months of the index cycle and to an accuracy of 0.1 kg and 1 cm using equipment that is regularly checked for accuracy. The women were then categorized into three groups as lean (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (BMI ≥30 kg/m^2). Three women with a BMI less than 18.5 kg/m^2 were excluded as they are classified as underweight.

Embryology and genetic screening

Fertilization with intracytoplasmic sperm injection (ICSI) was mandated for all PGS cases. The resulting embryos were cultured in a sequential system (G-1 PLUS/G-2 PLUS: Vitrolife, Goteborg, Sweden) at 6% CO_2, 5% O_2, 89% N_2 at 37°C in groups (50 µl-drops of up to four embryos) from day 1 to day 3, and after day 3 they were cultured in individual 10-µl drops. Embryo biopsy using laser (Octax LaserShot, Octax, Herborn, Germany) was carried out between day 4 (blastomere) or day 5/6 (trophectoderm) of development depending on the day of oocyte retrieval and embryo developmental stage. Only embryos with between 12 and 32 cells and significant compaction on day 4 or an expanding blastocyst on day5/6 with both inner cell mass and trophectoderm of grade A or B (Gardner et al., 2000) were biopsied and cryopreserved. Women undergoing a Monday oocyte retrieval generally underwent a day 4 embryo biopsy (Friday) so as to minimize weekend embryology workload; this approach has been reported to maintain embryo viability or implantation compared with blastocyst biopsy (Zander-Fox et al., 2014). After cell biopsy, embryos were vitrified (Rapid-I system: Vitrolife) pending PGS analysis.

Biopsied cells were washed and placed into sterile 0.2 ml polymerase chain reaction tubes containing 2 µl phosphate buffered saline (Sigma Chemical Company, St Louis, MO, USA) and stored at –20°C until whole genome analysis. This was carried out using SurePlex DNA

Amplification System (BlueGnome, Cambridge, UK) according to the manufacturer's instructions. A positive (genomic DNA) and negative (amplification mix only) control was also included in each amplification run. The whole genome analysis products were then processed using the BlueGnome 24sure cytochip protocol, which uses array CGH technology (Gutiérrez-Mateo et al., 2011).

Cryopreserved embryo transfer

Patients with a regular menstrual cycle underwent hormonal tracking to time embryo transfer, without any hormonal support. Anovulatory patients were placed on either ovulation induction treatment (clomiphene citrate or recombinent FSH), or managed with an artificial hormone replacement cycle depending on clinical circumstances. Artificial hormone replacement cycles consisted of a minimum of 14 days of oestrogen replacement (oestradiol valerate 2 mg three times a day, Bayer Australia) before starting progesterone treatment (Crinone 8% vaginal gel twice a day, Merck Australia) once the endometrium was at least 7 mm thick on ultrasound assessment. Potentially ovular patients or those women receiving long down regulation hormonal treatment for adenomyosis (Niu et al., 2013; Tremellen and Russell, 2011) were pre-treated with pituitary down-regulation (Leuprolide acetate, Abbott Australia or goserelin acetate, AstraZenca Australia) before starting ostrogen or progesterone replacement, and all women on artificial hormone replacement cycles continued hormonal support until 11 weeks' gestation. On the day of embryo transfer, the embryo was warmed and placed into EmbryoGlue (Vitrolife) for at least 1 h before placement into the uterine tract using ultrasound guided embryo transfer.

Pregnancy determination and birth outcomes

All patients had determination of serum beta-HCG 16 days after ovulation (unless menstruation began before this date). Biochemical pregnancy was defined as two rising serum beta-HCG concentrations greater than 5 IU/l. A pregnancy that failed before a fetal sac could be seen on ultrasound was defined as a biochemical miscarriage. A clinical pregnancy was defined as the presence of a fetal sac per embryo transfer and a clinical miscarriage was defined as the presence of a gestational sac with no fetal heart movement. A clinically viable pregnancy was determined as the presence of at least one gestational sac with fetal heart motion present. Data of live births, gestation at delivery, fetal weight, stillbirths, neonatal deaths and congenital abnormalities were recorded, as well as any maternal complications, as per local legislative requirements.

Ethical approval

Institution review board approval to collect and analyse these data was obtained from the Southern Adelaide Clinical Human Research Ethics Committee on 18 May 2015 (App: 203.15). All participants had previously provided written permission for their notes to be accessed for the purposes of low-risk retrospective audits such as this study, as per Australian National Health and Medical Research Council ethical guidelines.

Statistical analysis

Differences in continuous variables between the three BMI groups were assessed by analysis of variance and post-hoc analysis, using the Bonferroni test, whereas differences in proportions were assessed using either chi-squared or Fisher's exact test. Correlations were carried out using pregnancy outcomes as the dependent variable when controlling for other potential cofounders, such as BMI group, age, duration of infertility, parity, number of previous IVF cycles, type of FET cycle (ovular, artificial cycle), day of embryo biopsy and cause of infertility. Regression analysis was used to analyse the potential effect of BMI group, age, duration of infertility, parity, number of previous IVF cycles, type of FET cycle (ovular, artificial cycle), day of embryo biopsy and cause of infertility that could bias pregnancy outcome results. All statistical analysis was carried out using Graphpad Prism 6 (Graphpad, La Jolla, CA, USA) and SPPS (IBM Corp., Armonk, NY, USA, 2011, version 23.0), with $P < 0.05$ being considered as statistically significant.

Results

The baseline characteristics of the 125 women divided into three BMI subgroups (lean, overweight, obese) are outlined in **Table 1**. No significant difference was found in maternal age or past obstetric history (gravida, parity) between the three BMI groups. The duration of infertility was significantly longer in the overweight and obese groups compared with the lean group ($P = 0.0025$), but no significant difference was found in the number of previous IVF attempts undertaken. The principal causes of infertility in the entire cohort were male factor (24%), combined male and female infertility (17.6%), unexplained infertility (15.2%), advanced maternal age (40 years and above) (12%), polylcystic ovary syndrome (PCOS) (8%), endometriosis (8%), tubal factor (7.2%) and other miscellaneous causes (8%). Infertilty related to PCOS (combining those women with pure PCOS-related female factor infertility and PCOS in the setting of combined male and female factor infertility) was significantly more common in the obese (32.2%) compared with the overweight (12.5%) and lean (10%) groups ($P = 0.017$). Only a minority of patients were current smokers (3.2%), and the number of smokers was not different between any of the BMI groups. The presence of significant chronic medical conditions in the study cohort was more common in the obese group: chronic depression ($n = 2$); non-insulin-dependent diabetes mellitus ($n = 2$); hypertension ($n = 2$); hypothyroidism ($n = 1$); trigeminal neuralgia ($n = 1$). The significant chronic medical conditions in the overweight group were as follows: hypothyroidism ($n = 3$) and von Willebrand disease ($n = 1$). In the lean group, the conditions were asthma ($n = 2$) and epilepsy ($n = 1$). All of these conditions were adequately treated at the time of starting fertility treatment and during early pregnancy. No participant had a history of miscarriage (three or more consecutive miscarriages) or a medical condition known to predispose to miscarriage.

Overall, 32% of embryo biopsies occurred on day 4, with the remainder occurring at the blastocyst stage (day 5/6, primarily Wednesday and Friday oocyte retrieval embryos). No significant difference was observed in the proportion of embryos biopsied on day 4 when comparing the lean (28.6%), overweight (33.3%) or obese groups (35.5%). Furthermore, the miscarriage rate for pregnancies resulting from day 4 (27.5%) and blastocyst biopsy (22.4%) were not significantly different.

All embryo transfers were conducted in a cryopreserved embryo transfer cycle, with the most transfers occurring in natural ovular cycles (59.2%), or through ovulation induction using clomiphene citrate

Table 1 – Characteristics of participants.

	Lean (BMI 18.5–24.9) (*n* = 70)	Overweight (BMI 25–29.9) (*n* = 24)	Obese (BMI ≥30) (*n* = 31)	*P*-value
Age (years)	36.1 ± 4.6	35.8 ± 4.0	36.2 ± 5.2	NS
Body mass index (kg/m^2)	21.9 ± 1.7	26.9 ± 1.4	34.7 ± 4.6	Not applicable
Gravida	1.27 ± 1.3	1.95 ± 1.6	1.55 ± 1.3	NS
Parity	0.45 ± 0.62	0.38 ± 0.5	0.42 ± 0.56	NS
Cause of infertility				
Male (%)	18.6	29.2	32.2	NS
Tubal (%)	8.60	4.20	6.50	
PCOS (%)	7.10	4.20	12.9	
Advanced maternal age (%)	15.7	4.20	9.70	
Endometriosis (%)	5.70	12.5	9.70	
Male and female (%)	15.7	20.8	19.3	
Unexplained/ other (%)	28.6	24.9	9.70	
Duration infertility (years)	2.0 ± 1.3	3.0 ± .8	2.8 ± 1.4	0.0025
Number of previously stimulated IVF cycles	2.1 ± 2.0	1.9 ± 2.0	2.5 ± 2.1	NS

Continuous variables are expressed as mean ± standard deviation, with statistical differences between groups assessed by analysis of variables. Differences in proportions (%) were assessed by Fisher's exact test. NS, not statistically significant; PCOS, polycystic ovary syndrome.

or recombinent FSH (16%). The use of artificial hormone replacement cycles, however, was more common in the overweight (29.1%) and obese (35.5%) groups compared with lean women (18.6%), principally as a result of an increased incidence of long-down regulation artificial hormone replacement treatment of adenomyosis (obese 22.6% of cycles, overweight 20.8% of cycles, lean 8.6%) and previous failure to ovulate using clomiphene citrate in the groups with a BMI exceeding 25 mg/kg^2.

Significant differences in pregnancy outcomes were observed between the BMI groups (**Figure 1**). The proportion of pregnancies that failed by the 8-week ultrasound increased from 14.2% in the lean group, 29.1% in the overweight group and 41.9% in the obese groups. This difference between the three BMI groups was highly statistically significant (**Figure 1**), (*P* = 0.001). The number of live births correlated with BMI group while controlling for other potential co-founders such as maternal age, duration of infertility, parity, number of previous IVF cycles, day of embryo biopsy, type of FET cycle and cause of infertility (*P* = 0.023).

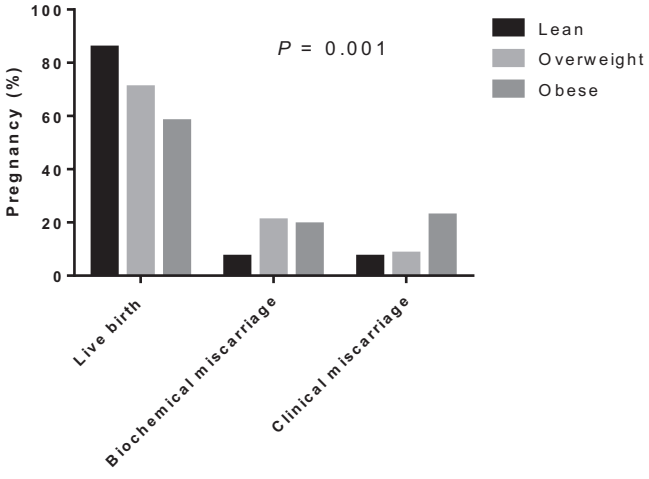

Figure 1 – Pregnancy outcomes according to body mass index classification.

A stepwise multiple regression was conducted to assess the effect of BMI group, age, duration of infertility, parity, number of previous IVF cycles, type of FET cycle (ovular or artificial cycle), day of embryo biopsy and cause of infertility to predict pregnancy outcome. Preliminary analysis was carried out to ensure no violation of the assumption of normality, linearity, homoscedasticity and multicollinearity. Two significant models emerged: model one consisted of BMI group alone (F [1122] = 11.46, *P* = 0.001 with an r2 = 0.08, beta = –0.29, t = 3.39, *P* = 0.001) and model two consisted of BMI group and FET (F [2121] = 8.4, *P* < 0.001 with an r2 = 0.11; with BMI recording a higher beta score of –0.25, t = 2.81, *P* = 0.006 compared with the type of FET used; beta = –0.20, t = 2.23, *P* = 0.028. In model 1 pregnancy outcome = 1.002 – (0.146) × BMI group, where BMI was coded as 1 = normal weight, 2 = overweight and 3 = obese. Eight per cent of the variance in live births was predicted by BMI alone, whereas 11% of the variance was predicted by BMI and type of FET. In model 2, pregnancy outcome = 1.1 – 0.122 (BMI group) – 0.075 (type of FET), with FET coded as 1 = natural ovulation; 2 = use of ovulation treatment code; 3 = artificial treatment cycle and 4 = long-down regulation treatment of endometriosis and ademonyosis with follow-up artificial hormone replacement therapy. For obese women, the likelihood of falling pregnant varied according to the method of FET with use of ovulation treatment code, artificial treatment cycle and long-down regulation treatment of endometriosis and adenomyosis with follow-up artificial hormone replacement therapy. Both BMI and type of FET were significant predictors. Importantly, an increase in weight and the use of an artificial FET cycle corresponds to a decrease in the likelihood of pregnancy outcome.

A sub-group analysis was carried out to examine the rate of pregnancy loss depending on both type of FET cycle used (ovular or artificial hormone replacement) and BMI classification. The overweight and obese groups were combined into a single group to increase the statistical power of this sub-analysis. As can be seen from **Table 2**, the overall rate of miscarriage in the overweight and obese group was significantly higher than seen in the lean group (36.4% versus 14.3%; *P* = 0.006). Interestingly the rate of miscarriage in the entire cohort was also significantly higher in women on artificial hormone replacement cycles than ovular cycles (45% versus 17%; *P* = 0.003), with this

Table 2 – Miscarriage rate according to body mass index status and type of endometrial preparation.

	Ovular cycles (*n* = 94), *n* (%)	Artificial hormone replacement cycles (*n* = 31) *n* (%)	*P*-value
Lean (BMI <25) Total (*n* = 70)	7/57 (12)	3/13 (23)	NS
Overweight and obese (BMI >25) Total (*n* = 55)	9/37 (24)	11/18 (61)	0.015
Overall miscarriage rate	16/94 (17)	14/31 (45)	0.003

Differences in proportions were assessed by Fisher's exact test. NS, not statistically significant.

difference being even more marked in the overweight and obese sub-group (61.1% versus 24.3%; *P* = 0.015).

A further sub-group analysis was conducted examining the effect of adenomyosis on pregnancy outcomes, as adenomyosis has previously been reported to increase miscarriage risk (Vercellini et al., 2014), and was more prevalent in the overweight and obese groups. The miscarriage rate in lean adenomyosis patients was not significantly higher than that seen in the lean non-adenomyosis (ovular) patients (16.6% versus 12.3%), nor were miscarriage rates significantly increased in the adenomyosis positive overweight and obese group compared with non-adenomyosis patients (50% versus 32.6%). The number of cases, however, included in this sub-group analysis is low (six cases adenomyosis lean group, 12 overweight and obese), making firm statistical conclusions impossible.

In two women, the transfer of a solitary embryo resulted in a probable monozygotic twin birth; however, for the purpose of this study, these pregnancies were assessed as a single live birth event. Furthermore, one pregnancy was terminated at 21 weeks for cleft palate. As this condition is normally associated with live birth and the pregnancy had reached more than 20 weeks' gestation, this pregnancy was assessed as a live birth.

Gestation at delivery did not significantly differ between the three BMI groups (lean 38.2 ± 1.6 weeks; overweight 39.2 ± 1.1 weeks; 37.6 ± 3.7 weeks). The mean birth weight in the overweight group, however, significantly exceeded that in the lean group (3578 ± 379 versus 3177 ± 610 g; *P* = 0.008), but no significant difference in birth weight was found compared with the obese group (3266 ± 851 g). Overall, six babies (4.8%) were born with a congenital anomaly (four in the lean group, one in the overweight and one in the obese group), making valid statistical comparisons between groups impossible.

Discussion

The results of our study clearly indicate that the chances of live birth after the transfer of a genetically normal embryo are significantly reduced in overweight women, especially those with a BMI in the obese range (BMI ≥30). Although previous studies examining the genetic status of miscarriage POC using traditional karyotyping had suggested a non-genetic cause for the reported increased risk of pregnancy loss in obese women, our study, as far as we know, is the first to examine this question using CGH-based preimplantation genetic screening. As CGH technology has superior diagnostic resolution, and is not affected by maternal cell contamination like POC karyotyping can be (Dhillon et al., 2014), we believe that the results presented in this paper provide a significant advance in our understanding of the potential underlying cause of increased miscarriage risk in obese women.

An increased risk of miscarriage in obese women who conceive naturally (Hahn et al., 2014; Lashen et al., 2004; Metwally et al., 2008) or with the aid of IVF (Bellver et al., 2003; Fedorcsák et al., 2004; Moragianni et al., 2012; Rittenberg et al., 2011) has been consistently reported; however, it is still unclear whether defects in the oocyte, embryo or uterine environment are primarily responsible for this reproductive deficit. The absence of a correlation between obesity and embryo aneuploidy (Goldman et al., 2015), plus an over-representation of euploid spontaneous abortions in POC from obese compared with lean women suggests a potential problem with uterine function rather than fetal genetics (Boots et al., 2014; Kroon et al., 2011; Landres et al., 2010). Furthermore, the Metwally meta-analysis (2008) of 16 studies and over 16,000 pregnancies reported a significantly increased risk of miscarriage in overweight and obese individuals compared with normal weight women (OR 1.67, CI 1.25 to 2.25), with this difference still being significant in obese recipients of donated oocytes (OR 1.52, CI 1.1 to 2.09). This finding suggests that the uterine milieu, rather than oocyte quality, is responsible for the increased rate of post-implantation loss in obese individuals. It should be acknowledged, however, that the absence of a genetic defect in a miscarriage does not necessarily mean that obesity cannot impair pregnancy through an alternative genetic mechanism, such as epigenetic modification of critical gene expression, as this is not assessed by CGH technology. Obesity has been reported to alter placental gene expression (Martino et al., 2016) and the early embryo metabolomic signature (Bellver et al., 2015), thereby raising the possibility of epigenetic mediated impairment of pregnancy outcomes.

A recent meta-analysis of pregnancy outcomes in donor oocyte IVF, primarily using young donors with low rates of embryonic aneuploidy, has reported that recipient BMI had no effect on miscarriage rates (Jungheim et al., 2013), thereby suggesting that obesity does not produce a sub-optimal uterine environment for pregnancy. This study is in direct contrast to the findings of the larger SART database study of 22,317 donor oocyte and recipient patients, in which morbid obesity was linked to a 67% increase in pregnancy loss (Provost et al., 2016). Interestingly, recipients in the Jungheim meta-analysis (2013) were on high-dose intra-muscular progesterone support, potentially masking any implantation deficit related to 'luteal insufficiency' in the obese cohort. The type of luteal support used by patients in the Provost (2016) study was not reported, but most likely reflects contemporary clinical practice where both vaginal progesterone alone, intramuscular progesterone, or both, are used, potentially explaining the differences in outcomes.

Previous reports have shown that obesity is associated with a reduction in progesterone production by the corpus luteum (Rochester et al., 2009; Tremellen et al., 2015), and that maternal obesity is associated with lower serum progesterone in early pregnancy (Goh et al., 2016). As progesterone plays a pivotal role in the maintenance of pregnancy, with low progesterone predicting subsequent pregnancy loss even before the onset of bleeding (Arck et al., 2008), it is possible that high-dose luteal progesterone supplementation may help rescue pregnancies in obese women. Although one-third of the obese cohort in our study also used progesterone hormone support, the intensity of luteal support used in our study (vaginal progesterone cream, Crinone)

is less than that used in patients included in the Jungheim et al. (2013) meta-analysis in which intramuscular progesterone was primarily used. Inadequate progesterone effect would help explain the significantly higher miscarriage rate seen in our overweight and obese women using vaginal progesterone support compared with ovular overweight and obese women. Interestingly, it has previously been reported that the use of vaginal progesterone (Crinone) support is associated with significantly lower live birth rates in women undergoing donor oocyte IVF treatment compared with intramuscular progesterone (Kaser et al., 2012). Although vaginal progesterone may be adequate to decidualize the endometrium, it is possible that the significantly lower levels of serum progesterone seen compared with intra-muscular progesterone therapy may still result in some reproductive impairment in overweight individuals. This impairment was not evident in our lean cohort in whom miscarriage rates were not significantly different between ovular women and those on artificial hormone replacement cycles, as has also been reported in a recent large randomized controlled study (Groenewoud et al., 2016).

It is presently uncertain how obesity mediates its negative effect on implantation and post-implantation events. A recent study has reported that obesity impairs decidualization, with both smaller implantation sites in pregnancy and a 50% reduction in the size of deciduomas created in a rodent experimental model of early implantation (Rhee et al., 2016). Another human study sampled endometrial tissue in the mid-luteal phase and reported an alteration in endometrial protein profile, especially endometrial haptoglobin, compared with lean counterparts (Metwally et al., 2014). As haptoglobin is associated with inflammation and plays a role in prevention of oxidative stress (Thomsen et al., 2013), it is possible that obesity-related inflammation and oxidative stress may be the underlying cause of miscarriage in obese women as oxidative stress has already been linked with early miscarriage (Agarwal et al., 2012). Progesterone is known to possess potent anti-inflammatory action, inhibiting the production of reactive oxygen species by activated leukocytes, while also enhancing production of protective antioxidants (Evans and Salamonsen, 2012; Hughes, 2012). Therefore, it is interesting to speculate that the high serum levels of progesterone observed with the use of intramuscular progesterone luteal support may help supress systemic oxidative stress, thereby protecting the fetus from oxidative damage. This mechanism would help explain why the Jungheim study (2013) reported no increase in miscarriage in obese recipients of donor oocytes receiving intra-muscular progesterone, whereas most studies looking at natural conception without luteal support do report a clear increase in miscarriage risk with obesity. Although this mechanism is currently entirely speculative, we believe that this hypothesis warrants further examination in future prospective studies.

As adenomyosis was more commonly observed in the obese and overweight groups, and has been linked with increased risk of miscarriage (Vercellini et al., 2014), it is possible that adenomyosis may play some role in the increased rate of pregnancy loss in obese women. This role is only likely to be minor, as BMI was still a significant determinant of miscarriage risk on regression analysis after controlling for adenomyosis status, plus miscarriage rates were not significantly different between adenomyosis positive and negative patients in the overweight and obese cohort.

We acknowledge that our study has several limitations. First, the overall study size is relatively modest, with only 24 and 31 of participants being overweight or obese, respectively. As Australian guidelines generally do not support the use of IVF in women above a BMI of 35 kg/m^2 (RANZCOG, 2016), however, this is a still a significant cohort for a single-centre study. Furthermore, despite the relatively small sample size, our study was adequately powered to detect a statistically significant difference in pregnancy outcomes because of the large impact that obesity has on miscarriage rates.

Our study was retrospective in nature, and therefore concerns always surround inaccurate or incomplete collection of data and potential bias. Pre-implantation genetic screening is not yet standard practice in our IVF unit, and is primarily used for patients of advanced maternal age or those patients who have undergone several previously unsuccessful cycles of treatment. As such, our study cohort is likely to be older and have had more fertility treatment than the average infertile patient in our unit. As no difference was found in maternal age or number of previous IVF cycles between the three BMI groups, we feel that these factors are unlikely to significantly bias the conclusions of the study. Furthermore, for patients who had undergone several previous cycles of IVF treatment, we only analysed their first PGS cycle, which resulted in a pregnancy, not subsequent pregnancy outcomes. Finally, as some studies have suggested that paternal obesity may adversely affect IVF outcomes (Petersen et al., 2013), it may have been useful to record and adjust for paternal BMI. Unfortunately, most men in our cohort did not have a current BMI on record, and this analysis was not possible. The Petersen study (2013), however, found no significant effect of paternal BMI on treatment outcomes when ICSI was used, as was the case in our entire study cohort. Furthermore, a more recent study has also failed to report any significant influence of paternal BMI on IVF outcomes (Schliep et al., 2015). Therefore, the influence of paternal BMI on pregnancy outcome is likely to be weak at best, and therefore we believe that our failure to control for male BMI is unlikely to be a significant weakness.

Embryoscopic assessment of the early embryo has previously revealed that many euploid miscarriages are caused by morphological defects (Philipp et al., 2003). Therefore, it is possible that obesity may mediate an increase in miscarriages because of non-aneuploid related defects in fetal development, rather than impaired uterine function. Diabetes, a condition more commonly seen in the obese population, is also a known risk factor for the development of congenital abnormalities (Wahabi et al., 2010). Poor glycaemic control in obese diabetic women could result in fetal morphological defects that in turn lead to miscarriage. We believe, however, that this is unlikely to be the major cause for increased miscarriage seen in our cohort, as only a small number of the overweight and obese group were diabetic, and all had adequate glycaemic control before starting IVF treatment.

In conclusion, to the best of our knowledge, our study provides the first evidence using an autologous oocyte derived euploid embryo model that miscarriage rates are increased in overweight and obese women owing to a non-genetic mechanism. Clearly, more research is required to identify the exact pathological mechanisms underlying these reproductive deficits so that more effective treatments can be offered to this disadvantaged obese group.

Acknowledgements

We would like to thank Kathryn Gebhardt for performing the majority of the embryo genetic analysis used in this study cohort, and for collecting some of the treatment outcome data.

ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 27 September 2016
Accepted 27 September 2016
Declaration: The authors report no financial or commercial conflicts of interest.

Keywords:
BMI
euploid
miscarriage
obesity
pre-implantation genetic screening

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