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ARTICLE

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

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Abstract 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.

KEYWORDS: BMI, euploid, miscarriage, obesity, pre-implantation genetic screening

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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 Laser-Shot, 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

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