Paternal body mass index is associated with decreased blastocyst development and reduced live birth rates following assisted reproductive technology

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Objective: To determine the relationship between paternal body mass index (BMI), embryo development and pregnancy, and live birth outcomes after assisted reproductive technology (ART).

Design: Retrospective analysis of ART cycles.

Setting: Major assisted reproduction center.

Patient(s): Three hundred five couples undergoing ART in a private fertility clinic.

Intervention(s): No intervention was undertaken in patients involved in this study.

Main Outcome Measure(s): Live birth outcomes and clinical pregnancy rates.

Result(s): No significant relationship between paternal BMI and early embryo development was found. However, increased paternal BMI was associated with decreased blastocyst development, clinical pregnancy rates and live birth outcomes.

Conclusion(s): To our knowledge, this is the first report linking increased paternal BMI and clinical pregnancy and live birth rates after ART treatment. Further work to elucidate the mechanisms involved is required. (Fertil Steril® 2010; 84–III–III. ©2010 by American Society for Reproductive Medicine.)

Key Words: Sperm, embryo, pregnancy, obesity, male fertility

Obesity and its health consequences are an increasing health burden. In the United States, the prevalence of obesity in young, reproductive-age men has tripled since the early 1970s (1). Similarly, in Australia more than 7 million adults are overweight or obese, and, according to the Burden of Disease and Injury in Australia study, high body mass was responsible for 7.5% of the total burden of disease and injury and is on the rise (2).

The potential role of male obesity in infertility essentially has been ignored until the last 2 to 3 years. This is surprising because 50% of reproductive-age males are overweight (2), and there are several examples of how paternal health around the time of conception affects the health of the offspring (3–5). There is also a known causal association between paternal age and significant medical conditions in the offspring such as autism (6). Similarly, children born to fathers of specific occupations have been shown to be at an increased risk for development of congenital abnormalities (7, 8). Furthermore, there is also an association of increased paternal body mass index (BMI) around the time of conception with an increase in the BMI of the offspring (9, 10).

Although the effect of paternal obesity on sperm function has been studied (11, 12) and systematically reviewed (13), there is limited information as to the effects of paternal obesity on embryo quality and subsequent pregnancy and live birth outcomes after ART treatment (14). Therefore, the aim of the current study was to determine whether or not there is an association between male BMI, fertilization, embryo development, and pregnancy and live birth rates after ART.

MATERIALS AND METHODS

Patients included in this study attended the infertility clinic, Repromed, Dulwich, South Australia, Australia, between January and May 2008. A total of 305 couples undergoing a fresh ART cycle were included in this study. Couples in which the female partner was <38 years of age (as outlined by the Reproductive Technology Accreditation Committee) at the time of oocyte collection were included to minimize the effect of maternal age as a confounding factor. Couples undergoing donor or frozen sperm treatment were excluded from the study. Frozen sperm was excluded because cryopreservation has been shown to induce sperm oxidative stress and DNA damage (15), which may result in poorer outcomes after ART. None of the men had any significant symptoms or signs of andrologic dysfunction. A retrospective chart review was performed to obtain the patients’ semen analysis, ART cycle outcomes, and paternal and maternal age. Approval was granted from Repromed’s Institutional Review Board. No identifying information was used in this study.

Paternal and Maternal Body Mass Index

Paternal and maternal height (meters) and weight (kilograms) were measured at the clinic before the treatment cycle and converted into BMI (kilograms per meter squared). Couples were classified according to the following paternal BMI ranges: normal weight BMI 20 to 24.9 kg/m², overweight 25 to 29.9 kg/m², obese 30 to 34.9 kg/m², and morbidly obese ≥ 35 kg/m².

Conventional Semen Analysis

Evaluation of sperm samples for the initial diagnosis before the treatment was performed and reported according to the World Health Organization (WHO) methods and criteria (16). After abstinence of 1 to 6 days, semen samples were collected by masturbation in sterile containers. When necessary, samples were transported to the laboratory maintained at room temperature. Samples were analyzed within 1 hour after collection in all cases and as necessary.
recommended by the 1999 WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction (16). Sperm motility was determined manually under >40 magnifications and expressed as a percentage of motile sperm in a given sample. Duplicate measures of 200 sperm were counted for motility assessment. Sperm concentration was determined with use of an improved Neubauer hemocytometer and applying the appropriate dilution factor. The percentage of morphologically normal sperm also was calculated by the Diff-Quik staining method as described by the 1999 WHO manual with use of strict morphology. Two hundred sperm were counted for motility assessment. Sperm concentration was determined with use of an improved Neubauer hemocytometer and applying the appropriate dilution factor. The percentage of morphologically normal sperm also was calculated by the Diff-Quik staining method as described by the 1999 WHO manual with use of strict morphology. Two hundred sperm were counted for each sample (16).

**Ovarian Stimulation**

All female partners in this study underwent controlled ovarian stimulation with use of recombinant FSH (Puregon; Schering-Plough, Sydney, Australia; Gonal-f; Serono, Sydney, Australia) and a GnRH antagonist. Follicle development was monitored with use of both ultrasound and blood levels of estrogen and P, and ovulation was induced by hCG administration (5,000 IU Pregnyl; Schering-Plough; 250 gm Ovidrel; Serono) when two or more follicles ≥17 mm in diameter were present (17). Luteal support was provided by a combination of daily vaginal P (Crinone 8%; Serono) and a single injection of 500 IU hCG (Pregnyl; Schering-Plough).

**Fertilization, Embryo Culture, Cleavage, and Grading**

Oocytes were collected at 36 hours following hCG and inseminated with use of either conventional IVF or intracytoplasmic sperm injection (ICSI) as described previously (18). Motile sperm were separated from semen samples with use of 40% and 80% Sperm Grad (Series III; Vitrolife AB, Gothenburg, Sweden). Insemination occurred between 4 and 6 hours after oocyte collection. For conventional IVF, oocytes were placed in 50-μL drops of fertilization medium with 30,000 spermatozoa. For ICSI, a single motile spermatozoon was selected and injected into the oocyte. Fertilization was assessed the following morning (16–18 hours after insemination) by the presence of two pronuclei and two polar bodies (2PN). All 2PNs were cultured in groups of two to four in G1 medium (Series III; Vitrolife AB). Cleavage-stage embryo morphology assessment was based on embryo cell number and the degree of fragmentation. For day 3 embryos assessed at 66 hours after insemination, a grade 1 embryo was considered to have eight cells and no fragmentation. Grade 1 and 2 embryos had a minimum of seven cells with <10% fragmentation and lacked multinucleated cells. Blastocyst quality was determined by the degree of blastocyst expansion and by assessment of the inner cell mass and trophoderm as previously described (18). All decisions for which embryos to transfer were based on morphology. Good-quality embryos were defined as those receiving scores of either grade 1 or 2 because these were considered suitable for freezing.

**Pregnancy Outcomes**

Biochemical pregnancy was determined when the serum β-hCG level of two consecutive blood tests was >20 IU from day 14 after ET. Clinical pregnancy was determined by presence of a viable fetal heartbeat on ultrasound examination 4 to 6 weeks after ET. Implantation rates were measured by the presence of fetal sac. Live birth outcomes including date of delivery, number of infants, weight, gender, and complications were received from the external obstetrician. Pregnancy loss was defined as any loss occurring between a positive β-hCG test and live birth.

**Statistical Analysis**

Retrospective analysis was carried out with use of SPSS software (SPSS Inc., Chicago, IL). Generalized linear modeling used either univariate analysis for male-only measures or multivariate analysis for cycle outcomes including both male and female BMI. Where appropriate, insemination method was used as a covariate. Pearson’s coefficient was used to assess linear relationships between embryo development and pregnancy rates with paternal BMI. A P value of .05 was considered statistically significant.

**RESULTS**

**Cohort Characteristics**

For this analysis patients were characterized into four groups based on paternal BMI (Table 1). There was no difference in paternal age among the BMI groups with the exception of paternal age among those with morbidly obese BMI, which was significantly lower than that of normal BMI patients.

**TABLE 2**

The diagnosis of infertility according to the categories of paternal body mass index.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Normal (n = 63)</th>
<th>Overweight (n = 148)</th>
<th>Obese (n = 62)</th>
<th>Morbidly obese (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male factor (%)</td>
<td>43.4</td>
<td>49.2</td>
<td>46.6</td>
<td>52.3</td>
</tr>
<tr>
<td>Endometriosis (%)</td>
<td>7.9</td>
<td>9.3</td>
<td>4.1</td>
<td>9.1</td>
</tr>
<tr>
<td>Tubal–pelvic disease (%)</td>
<td>9.2</td>
<td>3.3</td>
<td>12.3</td>
<td>9.1</td>
</tr>
<tr>
<td>Ovulation–ovarian defect (%)</td>
<td>17.1</td>
<td>6.0</td>
<td>8.2</td>
<td>6.8</td>
</tr>
<tr>
<td>Unexplained (%)</td>
<td>0.0</td>
<td>0.5</td>
<td>1.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Other (%)</td>
<td>22.4</td>
<td>31.7</td>
<td>27.4</td>
<td>22.7</td>
</tr>
</tbody>
</table>

*Note: P values are not statistically significantly different for all comparisons.*

across the groups. Similarly, there was no difference in maternal age. However, although there was no difference in maternal BMI for the male groupings of normal, overweight, or obese, there was a significantly higher maternal BMI in the morbidly obese male BMI group compared with the normal-weight group (P < .01). For all four groups the percentage of patients seeking treatment for male infertility was similar (Table 2), as was the percentage of cycles that used ICSI as the fertilization method. Couples with morbidly obese men had gone through more previous cycles compared with the other BMI groups (Table 1). The percentage of patients within the respective BMI categories was 20.7%, 48.5%, 20.3%, and 10.5%.

**Paternal Body Mass Index and Conventional Semen Analysis**

Sperm concentration for the normal-weight group was significantly higher than for the three other groups (78.8 ± 12.1, 57.8 ± 4.3, 50.4 ± 6.4, 58.2 ± 13.1 10^6 sperm/mL, respectively) (P < .05). In contrast, progressive motility only was reduced significantly in men who were morbidly obese (47.7% ± 2.6%, 46.0% ± 1.5%, 46.9% ± 2.2%, 32.6% ± 3.9%, respectively) (P < .05). There was no effect of male BMI on the percentage of normal sperm morphology (15.1% ± 1.8%, 13.4% ± 1.1%, 14.0% ± 1.9%, 13.9% ± 3.6%, respectively).

**Paternal Body Mass Index and Fertilization Rates and Embryo Development in Vitro**

Fertilization rates, either overall or after conventional IVF or ICSI separately, were not altered by paternal BMI. In this study it was not possible to establish the effects of morbid obesity state on conventional IVF fertilization because only three patients had insemination method in the morbidly obese group (Table 3).

There was no effect of paternal BMI on day 3 cleavage rates or the morphologic grade of embryos whether conventional IVF or ICSI was the insemination method. In contrast, there was a significantly linear reduction in expanded blastocyst development rates per 2PN with increasing paternal BMI (P < .05) (Table 3).

**Paternal Body Mass Index and Pregnancy and Live Birth Outcomes**

There was a highly statistically significant linear reduction in pregnancy rate with increasing paternal BMI from normal to obese men (P < .01). This was further decreased for men who were morbidly obese; however, this latter reduction in pregnancy rate was a combined effect of both high paternal and maternal BMI (P < .01). Implantation rate (as measured by the presence of fetal sac) decreased as paternal BMI increased (Table 4). Conversely, there was an overall increase in pregnancy loss with increasing paternal BMI (Table 4). The method of insemination did not alter these outcomes significantly.

Similarly, viable ongoing pregnancy rates (as measured by the presence of fetal heart at the 4- to 6-week scan) decreased as paternal BMI increased (Table 4). Live birth rates were reduced significantly by increasing paternal BMI (P < .05) (Table 4). There was no difference in gestation length (range from 36.2 ± 0.6 to 36.4 ± 0.4 weeks) or infant weight (range 3,054 g to 3,212 g) across the four groups nor any effect on sex ratios.

**DISCUSSION**

This study represents the first attempt in the literature to determine the effect of paternal obesity on embryo development, pregnancy, and live birth outcomes after ART. This study demonstrates that, as paternal BMI increases, several outcomes are impaired including reduced blastocyst development, reduced clinical pregnancy rates, and decreased live birth outcomes, as well as increased risk of miscarriage.
The first surprising result was that 79.4% of male patients in our study were either overweight, obese, or morbidly obese compared with only 20.5% who were in the normal-weight category. This was highly surprising especially given that the selection criteria for this study were primarily based around the female age (<38 years). This figure is also higher than the general population estimates where in Australia 62% of the population aged 18 years and over are either obese or overweight (19). This provides evidence that overweight and obese men are perhaps more common in a subfertile population. Of interest, morbidly obese patients tended to have more ICSI treatment compared with the other three groups. This may be due to the decreased sperm motility in this group.

It now has been demonstrated that the incidence of oligospermia triples from 5.32% in men of a normal BMI to 15.62% in obese men (20). Similar to other studies, our study also showed that sperm count declined in men who were overweight, obese, or morbidly obese. It is generally accepted that obesity can be associated with reduced sperm concentrations; however, this relationship has been challenged because obese men still may have normal sperm concentrations and there is a lack of a linear relationship, reviewed by Hammoud et al. (21).

Studies of the relationship between male obesity and sperm motility have been less certain and show conflicting results. Although some studies have shown no association between paternal BMI and the percentage of motile sperm (11, 22), others have shown paternal BMI to be negatively correlated with motile sperm count (12). This may be due to the different classifications used to determine BMI groupings between studies. In our study we found an effect on sperm motility only in men who were classified as morbidly obese.

The majority of studies investigating the relationship between male obesity and sperm morphology have reported no relationship (11, 23–27). Similarly, our study also found no relationship between sperm morphology and BMI. Therefore, it appears there are no increases in major structural abnormalities with increasing BMI groupings between studies. In our study we found an effect on sperm motility only in men who were classified as morbidly obese.

Our data in relation to embryo quality suggest no correlation between paternal BMI and cleavage-stage embryo development up to day 3. This is perhaps not surprising because it is generally accepted that the paternal genome is not activated until the fourto-eight-cell stage in humans, and therefore the influence of paternal BMI on sperm health and function would be limited before this stage (28). In contrast, at the later stages of embryo development after activation of the embryonic genome, our data showed a significant decrease in the proportion of expanded blastocysts (per 2PN) as paternal BMI increased. This is consistent with other studies, where other paternal factors such as sperm DNA damage similarly have shown no association with early embryo development (29–32) but are associated negatively with blastocyst development (33). Of interest, there have been two recent studies demonstrating that increased paternal BMI is associated with increased sperm DNA damage (12, 26). Therefore, we hypothesize that the decrease in blastocyst development observed in our study may be due to increased DNA damage.

We have demonstrated that paternal obesity at the time of conception is associated with reduced pregnancy rates and live birth rates after ART treatment. This observation is consistent with a recent study on IVF outcomes and epidemiologic studies where obese fathers have been shown to have a decreased chance of fathering a pregnancy (14, 34–39). However, unlike a recent study by Keltz et al. (14), we found that this relationship also exists for patients having ICSI. The reason for this difference may be due to the higher number of embryos transferred in the overweight group in the study by Keltz et al. (14), which, unlike our study where the vast majority were single ETs, may have masked the effects of paternal obesity on pregnancy outcomes. Epidemiologic studies show that this decrease is not mediated by sexual dysfunction in heavier men (35), perhaps suggesting that sperm molecular mechanisms are more likely to be the cause.

The effect of maternal obesity on fertility has been described in several studies in recent years, reviewed by Robker (40). Our results coupled with results in animal models (26, 41) suggest that equally paternal obesity affects fertility and pregnancy outcomes. In addition in our study, it is clear that a male partner with a very high BMI was significantly more likely to seek ART treatment with an obese female partner. However, none of the studies examining the effects of maternal BMI on pregnancy outcome and IVF have accounted for paternal BMI. Our study therefore highlights a likely confounding factor and possibly may explain, at least in part, some of the discrepancies reported in recent reviews in relation to maternal BMI effects (40).

Finally, this study represents the first steps in determining the effects of paternal obesity on subsequent reproductive health beyond sperm parameters including embryo, pregnancy, and live birth outcomes. Larger multicenter studies are required to confirm the results observed in this study. However, our own enquiries have discovered that the majority of units do not capture this information routinely. We therefore would encourage ART units to consider adding male weight and height to their standard assessment parameters. Clearly this study highlights the importance of determining the effect of weight loss on paternal reproductive health and fecundity.

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REFERENCES