Is maternal obesity related to semen quality in the male offspring? A pilot study

C.H. Ramlau-Hansen1,2,4, E.A. Nohr3, A.M. Thulstrup1, J.P. Bonde1, L. Storgaard1 and J. Olsen2

1Department of Occupational Medicine, Aarhus University Hospital, Aarhus Sygehus, Norrebrogade 44, Building 2C, DK-8000 Aarhus C, Denmark; 2Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA 90095, USA; 3Department of Epidemiology, Institute of Public Health, University of Aarhus, 8000 Aarhus C, Denmark
4Correspondence address. Tel: +45 89494290; Fax: +45 89494260; E-mail: craha@as.aaa.dk

BACKGROUND: Obesity is a strong predictor of fecundity and maternal obesity may well program semen quality during pregnancy, but to our knowledge, no published studies have evaluated this hypothesis. METHODS: From a Danish pregnancy cohort established in 1984–87, 347 out of 5109 sons were selected for a follow-up study conducted from February 2005 to January 2006. Semen and blood samples were analyzed for conventional semen characteristics and reproductive hormones and related to information on maternal pre-pregnant body mass index (BMI) that was available for 328 men. Of these, 34 were sons of underweight, and 25 sons of overweight, mothers. RESULTS: Inhibin B decreased with increasing maternal BMI ($P = 0.04$) and the point estimates for sperm concentration, semen volume, percent motile sperm, testosterone and FSH suggested an impaired reproductive status among sons of overweight mothers, but none of the trends were statistically significant. CONCLUSIONS: The results suggest that there may be an effect of high maternal BMI on the sons’ semen quality, but the study had only enough power to justify a critical evaluation of the hypothesis in a larger study.

Keywords: body mass index; prenatal exposure; reproductive hormones; semen; sperm count

Introduction
Fat is a hormonal active tissue, and overweight and obesity are associated with reduced fecundity in both women and men (Diamanti-Kandarakis and Bergiele, 2001; Gosman et al., 2006; Sallmen et al., 2006; Ramlau-Hansen et al., 2007a). Men’s body mass index (BMI) is reported to be associated with semen quality as well as altered levels of reproductive hormones (Jensen et al., 2004; Fejes et al., 2005, 2006; Magnusdottir et al., 2005; Kort et al., 2006).

Maternal overweight may have a programming effect on semen quality during fetal life, if a higher level of estrogen exposure interferes with the hormonal control of the development of the male fetal urogenital organs (Sharpe and Skakkebaek, 1993). Lipophilic persistent organic pollutants, such as polychlorinated biphenyls (PCBs), accumulate in adipose tissue, are found in both the maternal and the fetal blood stream (Jaraczewska et al., 2006; Jimenez et al., 2006) and provide an alternative mechanism to the programming hypothesis. Some environmental chemicals correlate positively with BMI (Magnusdottir et al., 2005) and poor semen quality (Hauser, 2006; Toft et al., 2006).

To our knowledge, no one has investigated the association between maternal BMI and semen quality in adult sons. We recently conducted a population-based follow-up study on the association between maternal smoking during pregnancy and semen quality, in which we found indication of an adverse effect of prenatal tobacco exposure on semen volume, sperm concentration and total sperm count (Ramlau-Hansen et al., 2007b). We use data from this cohort to examine the association between maternal pre-pregnant BMI and semen quality and levels of reproductive hormones in the offspring.

Materials and Methods
Population
The participants were sons of mothers who were recruited to the ‘healthy habits for two’ (HH42)-cohort during their pregnancy from April 1984 to April 1987. This study took place in two Danish municipalities (Aalborg and Odense), and 11 980 women with singleton pregnancies (more than 80% of all invited) participated. They provided information on lifestyle factors during pregnancy and health-related characteristics by filling out a self-administered questionnaire handed out by the midwives around the 36th week of gestation and returned in sealed envelopes to the university’s research department within a couple of weeks. Data on pre-pregnant heights and weights were entered in the women’s antenatal records by their general practitioners at the first routine antenatal care visit (Olsen...
et al., 1989) and later extracted from medical files together with birth data. Sons, who were alive and living in Denmark by December 2004, were identified in the Danish Civil Registration System (n = 5109).

Since the study was primarily designed to examine the association between prenatal smoking exposure and adult semen quality (Ramlau-Hansen et al., 2007b), the participants were selected according to levels of maternal smoking during pregnancy. A total of 716 men were invited to take part in the study, and 347 (48.5%) men gave consent and participated. Of 100 men that declined participation by mail or telephone, 82 provided some information on their health. There was no difference in the proportion of men with diseases of the reproductive organs (including cryptorchidism and hypospadias) between participants and non-participants. Information on maternal pre-pregnant BMI was available for 328 (94.5%) mothers, and their sons form the study group for this study.

The selected participants were 18 to 21 years of age and received economic compensation (~85 USD) for taking part in the study. Men with severe handicaps or congenital syndromes, such as spastic paraplegia or Down’s syndrome as well as men with metabolic diseases or psychiatric disorders were not invited to take part in the study. The study was approved by the Regional Ethics Committee (reg. number 20040174) and participation was made conditional on written informed consent. For further information on the selection and enrolment procedure see Ramlau-Hansen et al. (2007b).

**Exposure data**

Maternal BMI was calculated as pre-pregnancy weight in kilograms divided by height in meters squared and classified according to the criteria used by the World Health Organization (World Health Organization, 2000) as underweight (BMI < 18.50 kg/m²), normal weight (BMI 18.50–24.99 kg/m²), overweight (BMI 25.00–29.99 kg/m²) or obese (BMI ≥ 30.00 kg/m²). Since we only had 25 sons of mothers with BMI above 25.00 kg/m², we combined overweight and obese mothers into one group, hereafter referred to as ‘obese’.

**End-points**

Data collection took place from February 2005 to January 2006. The participants were instructed to provide the semen sample by masturbating into a plastic container at home after a minimum of 48 h of abstinence. The containers were then to be kept close to the body during transportation to avoid cooling and brought to a mobile laboratory where a trained medical laboratory technician performed the initial semen analysis. Blood samples were taken between 7:25 a.m. and 7:15 p.m. (medium time 1:10 p.m.). The participants completed questionnaires on their reproductive experience, medical and lifestyle factors, time and date of the preceding ejaculation and spillage during the collection.

**Semen analysis**

Semen analyses were performed blinded to maternal BMI and any other prenatal exposures. Semen volume was estimated by its weight (1 g = 1 ml). Sperm motility and sperm concentration was assessed as described in ‘WHO Laboratory Manual for the Examination of Human Semen-Cervical Mucus Interaction’ (World Health Organization, 1999). Examination of 83.2% of the samples was initiated within the first hour at which time it has been shown that the motility is stable (Makler et al., 1979), and examination of 99.7% of the samples was initiated within 2 h. Sperm morphology was determined using the strict criteria (Kruger et al., 1988). The laboratory took part in the Nordic ESHRE QC external quality control program, and all control tests were within the limits set by ESHRE.

**Testicular volume**

The participants were instructed to measure their testicular volumes themselves using a Prader orchidometer at the study site. In a study performed to validate this method, we found among 58 sperm donors and sperm donor candidates that self-measuring of testicular volumes by a Prader orchidometer was a reasonable valid method compared with measurements obtained by an experienced examiner (Ramlau-Hansen et al., 2007c). We calculated the mean value for both tests, and information of testicular volume was available for 195 (59.5%) participants.

**Analysis of serum samples**

After centrifugation, serum was stored at ~ 80°C for a maximum of 16.5 months until analysis. Serum samples for testosterone, estradiol, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were analyzed by Avida Centaur (Bayer Healthcare). Sex hormone binding globulin (SHBG) concentrations were determined using IMMULITE (DPC), and inhibin B was measured by a commercially available enzyme-linked immuno-sorbent assay (Oxford Bio-Innovation Ltd., Oxford, UK) according to the manufacturer’s instructions. The blood samples were analyzed blinded to maternal BMI and as single measurements in random order over a short period of time. The detection limits and total (intra- and inter-assay) CVs for the immunoassays were: testosterone: 0.35–52.1 nmol/l, < 7.7%, estradiol: 25.7–3670 pmol/l, < 12.4%, inhibin B: 15.0–1000 pg/ml, < 7%, FSH: 0.3–200 IU/l, < 4.0%, LH: 0.07–200 IU/l, < 3.9%, and SHBG: 0.02–180 mol/l, < 6.7%. The concentration of inhibin B in one sample was below the detections limit for the specific assay (15.0 pg/ml), and the concentration of inhibin B was therefore arbitrarily set to 14.0 pg/ml, before statistical analyses were performed.

The inhibin B samples were analyzed at the Laboratory of Reproductive Biology, University Hospital of Copenhagen, Denmark, and all other samples were analyzed at Department of Clinical Chemistry, Aarhus University Hospital, Denmark.

**Statistical analysis**

Crude median, 25th (p25) and 75th (p75) percentiles were calculated for all outcome variables. For each of the outcome variables, we performed multiple linear regression with maternal BMI group as the main exposure and sons of normal weight mothers (BMI 18.50–24.99 kg/m²) as reference. When we tested for trend, BMI group was entered as a continuous explanatory variable, using sons of underweight mothers (BMI < 18.50 kg/m²) as a starting point.

Data on all outcome variables, with exception of percentage of motile sperm and inhibin B, were cubic-root transformed to obtain a more symmetric distribution of residuals. Data on percentage of motile sperm were logit-transformed. The back-transformed means were presented with 95% confidence intervals (CIs) and were adjusted for minutes from ejaculation to analysis (continuous).

We also dichotomized outcomes into low sperm concentration (< 20 million/ml), low inhibin B (< 25 percentile) and high FSH (> 75 percentile) and used logistic regression to calculate the adjusted odds ratios (ORs) in relation to maternal BMI, using the sons of normal weight mothers as reference.
Participants, who reported spillage during sampling \( (n = 80) \), were excluded from all statistical analysis on semen volume and on total sperm count. All statistics were performed by using ‘Intercooled Stata 8.2’ (Stata Corporation, Texas, USA). A two-tailed \( P \)-value of less than 0.05 was considered statistically significant in all tests.

### Results

There was a tendency towards higher birthweight among men of overweight mothers in comparison with men of normal weight mothers \( (P = 0.14) \), and sons of overweight mothers...
had themselves a higher BMI as adults compared with men of normal weight mothers (P = 0.04) (data not shown).

Sons of overweight mothers more often had their semen and blood samples collected in the summer period than sons in the other groups (80% versus 59–62%) (data not shown).

There were no significant differences in the reported length of abstinence between the three groups (sons of underweight, normal weight and overweight mothers). The back-transformed mean days were 2.5, 2.7 and 2.8 days, respectively. We used a dichotomized abstinence variable in the statistical analysis (less or equal to 48 h or above 48 h), and there was no statistically significant difference between the proportion of participants with an abstinence time less or equal to 48 h between the groups (38%, 32% and 44%, respectively).

The crude median sperm concentration among sons of overweight mothers (n = 25) was 28 (p25–75: 20–76) million/ml in comparison with 37 (p25–75: 19–85) million/ml among sons of normal weight mothers (n = 269), which is equivalent to a 29% (statistically non-significant) difference, but most of the difference disappeared after transformation (Table 1).

On the other hand, sons of overweight mothers (n = 15) had the highest total sperm count compared with sons of normal weight mothers (n = 205) (adjusted mean 41% higher but statistically non-significant). The differences between the two groups (sons of, respectively, normal weight and overweight mothers) for the other semen outcome variables, testicular volume and blood outcome variables were about or less than 10% lower for the latter group, and all were statistically non-significant (Table 1).

We tested for trend across BMI strata by entering BMI group as a continuous explanatory variable in the multiple regression models and using sons of underweight mothers as the reference. Only the trend test for inhibin B was statistically significant (P = 0.04).

None of the ORs for low sperm concentration (<20 million/ml), low inhibin B (<25 percentile) or high FSH (≥25 percentile) in sons of overweight and underweight mothers were statistically significantly different from the risk in the reference group of sons of normal weight mothers (Table 2).

Discussion

This study indicates that there could possibly be an association between maternal BMI and semen quality and level of reproductive hormones in the male offspring, but this small pilot study had no power to identify moderate associations as indicated by the confidence limits.

Maternal obesity in pregnancy may be an indicator of an increased fetal exposure to estrogen due to decreased SHBG levels (resulting in relatively more bioavailable estrogen) and an increased conversion of androgens to estrogens (Pettigrew and Hamilton-Fairley, 1997). According to the ‘estrogen hypothesis’ (Sharpe and Skakkebaek, 1993), increased prenatal exposure to estrogens may lead to male reproductive disorders like testicular cancer, cryptorchidism, hypospadias and low sperm count. The ‘estrogen hypothesis’ has been evaluated by numerous studies addressing other objectives and these do not provide strong evidence for the hypothesis, with testicular cancer as an exception (Storgaard et al., 2006). So far, very few studies have been explicitly designed to examine the ‘estrogen hypothesis’ (Storgaard et al., 2002; Swan et al., 2005; Jensen et al., 2007). There are, however, other possible pathways that may link maternal obesity to impaired semen quality.

A possible decrease in semen quality over time has been much debated, and geographical differences in semen quality are evident (Swan et al., 2000). These differences could correlate with an increasing amount of body fat in fertile women, since the prevalence of obesity has increased at least since the 1960s in most Western countries and in the United States with large variations between the countries (Flegal et al., 2002; Silventoinen et al., 2004).

Strengths of our study include use of data on maternal pre-pregnant height and weight collected by the general practitioners at the first antenatal care visit where the woman additionally was weighed to provide an early pregnancy weight. This limits underestimation of pre-pregnancy BMI, which is often a problem when using purely self-reported data (Jalkanen et al., 1987). Also, we find it unlikely that any underestimation is associated with the future reproductive status of the sons. Our participation rate (48.5%) was rather high for semen quality studies, but not high enough to exclude selection bias. In order for this to cause selection bias, it had to be related to pre-pregnant BMI, which is unlikely. The source population is young, most had no reproductive experience and they were not aware of the exact hypothesis to be evaluated.

There was a tendency towards decreasing point estimates for semen volume and sperm concentration with increasing

---

Table 2: Low sperm concentration, low inhibin B and high FSH according to maternal pre-pregnant BMI (kg/m²)

<table>
<thead>
<tr>
<th></th>
<th>BMI &lt;18.50 (n = 34)</th>
<th>BMI 18.50–24.99 (n = 269)</th>
<th>BMI ≥25.00 (n = 25)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration, &lt;20 million/ml</td>
<td>6 (18)</td>
<td>68 (25)</td>
<td>6 (24)</td>
<td>0.58 (0.23, 1.49)</td>
</tr>
<tr>
<td>Inhibin B, &lt;25 percentile</td>
<td>7 (21)</td>
<td>67 (25)</td>
<td>9 (36)</td>
<td>0.72 (0.29, 1.76)</td>
</tr>
<tr>
<td>FSH, ≥75 percentile</td>
<td>10 (29)</td>
<td>66 (25)</td>
<td>7 (28)</td>
<td>1.28 (0.58, 2.83)</td>
</tr>
</tbody>
</table>

*OR adjusted for: maternal age (<27 years, >27 years) and maternal smoking during pregnancy (yes, no). Sperm concentration was additionally adjusted for abstinence time (<48 h, >48 h), and inhibin B and FSH were additionally adjusted for time of day for blood sampling (06.00 a.m.–12.00 p.m., >12.00 p.m.). Maternal age <27 years, no maternal smoking, >48 h of abstinence time and blood sampling between 06.00 a.m. and 12.00 p.m. were chosen as reference.*
maternal pre-pregnant BMI. Since total sperm count is derived by multiplication of these variables, it may seem odd that there was no tendency towards decreasing point estimates for this combined variable, but the reason is the exclusion of participants who reported spillage during semen sampling from the analyses on both semen volume and total sperm count. Excluded sons of normal weight mothers tended to have a higher sperm concentration than non-excluded sons of normal weight mothers, whereas excluded sons of, respectively, underweight and overweight mothers tended to have a lower sperm concentration than non-excluded.

Because of the limited number of participants of underweight and overweight mothers, we could only adjust for very few potential confounders. For example, we did not control for men’s individual BMIs, adult male smoking, season or diseases of the reproductive organs, although these factors have been shown to be associated with semen quality and levels of reproductive hormones.

In conclusion, the results observed in this pilot study may indicate a small to moderate effect of maternal pre-pregnant BMI on semen quality in sons, but the study had no power to provide more than suggestive evidence for such an effect. We encourage others who have the necessary data to follow-up on this important hypothesis.

Acknowledgements

We thank Joan Dideriksen for her important work with collecting the samples and performing the initial semen analysis. The study was supported by the Health Insurance Foundation (grant numbers 2004B137, 2005B081 and 2006B107), the Danish Medical Research Council (grant numbers 22-03-0200, 22-04-0271 and 271-05-0760), the Augustinus Foundation (grant number 05-2620), the Knud Højgaard Foundation (grant number 37.065), the Fulbright commission, the Simon Fouger Hartmanns Family Foundation, the Aase and Ejnar Danielsens Foundation, the University of Aarhus Research Foundation, and the Biomedical Laboratory Scientist Education and Research Fund.

References

Submitted on May 9, 2007; resubmitted on June 8, 2007; accepted on June 19, 2007.