Ovarian stimulation leads to shorter stature in childhood

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BACKGROUND: We aimed to determine whether children conceived with ovarian stimulation alone (OSA) would differ phenotypically and biochemically from naturally conceived of fertile and subfertile parents.

METHODS: Healthy pre-pubertal children aged 3–10 years, born at term, after singleton pregnancies were recruited in Auckland (New Zealand) and were allocated into three groups: (i) children conceived following OSA and naturally conceived children of (ii) subfertile and (iii) fertile parents. Anthropometric, endocrine and metabolic parameters were recorded. Children’s heights and body mass index (BMI) were expressed as standard deviation scores (SDS) and corrected for genetic potential (i.e. parental height or BMI).

RESULTS: Three hundred fifty-two children were studied: 84 OSA subjects and 268 naturally conceived controls consisting of 54 children of subfertile parents and 214 children of fertile parents. Children of subfertile and fertile parents did not differ in measured outcomes. Overall, OSA children were shorter than children of both subfertile (SDS: −0.08 ± 0.09 versus 0.32 ± 0.07; P = 0.001) and fertile (SDS: −0.08 ± 0.09 versus 0.45 ± 0.10; P = 0.004) parents when corrected for genetic height potential. OSA boys were shorter than boys of subfertile (SDS: −0.18 ± 0.14 versus 0.42 ± 0.16; P = 0.03) and fertile (SDS: −0.18 ± 0.14 versus 0.35 ± 0.08; P = 0.01) parents. There was also a trend towards OSA girls being shorter than girls of subfertile parents (P = 0.06), but not significantly shorter than those of fertile parents (P = 0.17). OSA children also had a lower corrected BMI SDS than children of subfertile (SDS: −0.90 ± 0.15 versus −0.37 ± 0.17; P = 0.06) and fertile (−0.90 ± 0.15 versus −0.34 ± 0.10; P = 0.008) parents. Among metabolic parameters, fasting glucose was lower in OSA children than that in children of fertile parents (4.62 ± 0.07 versus 4.81 ± 0.04; P = 0.006).

CONCLUSIONS: Conception after OSA was associated with shorter stature, particularly in boys, compared with naturally conceived children of fertile and subfertile parents.

Key words: child / clomiphene / height / subfertility / ovarian stimulation

Introduction

Almost one in seven couples have difficulty conceiving, with many going on to seek fertility treatment (Oakley et al., 2008). It is estimated that 2.5–7% of children in Western countries are conceived with the help of ovarian stimulation alone (OSA) (Schieve et al., 2009; de Mouzon et al., 2010) compared with 1–4% conceived through IVF (de Mouzon et al., 2010). Most fertility treatment utilizes ovarian stimulation; either alone or as part of the process of IVF (Berker et al., 2011). Ovarian stimulation is widely used in the treatment of infertility and the majority of women who undergo OSA receive either clomiphene citrate and/or follicle-stimulating hormone (FSH), often in conjunction with timed intercourse or intrauterine insemination (Berker et al., 2011).

There is evidence that IVF children are phenotypically different from naturally conceived children, having for example, higher rates of congenital abnormalities (Kallén et al., 2010) and rare imprinting disorders (Manipalviratn et al., 2009). When childhood stature has been examined, several studies have found that IVF children were taller than naturally conceived children (Saunders et al., 1996; Pruksananonda, 2001; Miles et al., 2007; Celen, et al., 2009; Makhoul et al., 2009; Green et al., 2010).

There is a growing recognition that parental subfertility may play an important role in some of the differences seen in children conceived...
after IVF, suggesting that parental subfertility should be considered in studies assessing outcomes on children conceived via IVF or with the help of other fertility treatments (Romundstad et al., 2008; Ceeelen et al., 2009; Wilkins-Haug, 2009; Rimm et al., 2011).

While several studies (reviewed elsewhere (Metwally and Ledger, 2011; Savage et al., 2011)) have assessed childhood outcomes after IVF conception, there is a marked paucity of studies assessing similar outcomes following OSA conception. Since IVF is a complex treatment combining several steps, including ovarian stimulation, embryo culture and embryo selection; it is unclear whether ovarian stimulation plays a role in the observed height differences between IVF children and those conceived naturally. Thus, we have studied phenotypic and metabolic measures of children conceived through OSA in an attempt to answer this question. We aimed to determine whether children conceived with the help of OSA would be phenotypically and biochemically different from naturally conceived children of fertile and subfertile parents.

Methods

Subjects

Only healthy pre-pubertal children aged 3–11 years, of European ethnicity, born at term (37–41 weeks gestation) after singleton pregnancies, and of birthweight appropriate for gestational age were included in the study. OSA subjects were recruited from a patient database of couples who attended for infertility treatment at Fertility Associates in Auckland between 2000 and 2006. Couples included in the study were those treated for unexplained infertility or mild male factor infertility. Children of women with a diagnosis of polycystic ovarian syndrome (PCOS; based on pelvic ultrasound) were excluded from the study, because of the possible association between maternal PCOS and phenotypic differences in their children (Sir-Petermann et al., 2007). Mothers of naturally conceived children did not undergo a pelvic ultrasound to rule out PCOS, but the children with a maternal history of PCOS were excluded from the study. The children of mothers who smoked, had a chronic illness, pre-existing or gestational diabetes or glucose intolerance during pregnancy were also excluded from the study.

OSA mothers received one of three fertility treatment regimens: (i) clomiphene citrate (Serophene; Merck Serono, NSW, Australia) for 5 days (dose: 25–100 mg day\(^{-1}\), mean dose 75 mg day\(^{-1}\)); (ii) clomiphene for 5 days (dose: 25–150 mg day\(^{-1}\), mean dose 75 mg day\(^{-1}\)) and FSH (Puregon, Merck Sharp & Dohme, Tipperary, Ireland) or Gonadotropin F (Merck Serono) alternative days (75–150 IU dose\(^{-1}\), mean total dose: 360 IU) or (iii) FSH daily (75–150 IU dose\(^{-1}\), mean total dose 375 IU). An ovarian response was monitored by serum estradiol, LH or ultrasound as appropriate; with hCG triggering (250 μg Ovidrel; Merck Serono) if an LH surge had not occurred prior to intrauterine insemination (82%) or timed intercourse (18%). All OSA children were conceived using the sperm from the mother’s partner, so that the offspring of donor sperm were not included in the study. Exclusion criteria for OSA children included those who had a known medical syndrome, a chronic illness, on treatment for 5 days (dose: 25–150 mg day\(^{-1}\)) or (iii) FSH daily (75–150 IU dose\(^{-1}\)). An ovarian response was monitored by serum estradiol, LH or ultrasound.

Study design

Standing and sitting height were measured using a Harpenden stadiometer. Children’s weight and body composition were assessed using dual-energy X-ray absorptiometry (DXA Lunar Prodigy 2000; General Electric, Madison, WI, USA). Each child had a bone age X-ray to assess biological age, based on birthweight data for ≏ 500 000 ‘healthy newborns’ in Sweden (Niklasson et al., 2001), which are also applicable to our study population. Children’s and parental heights were transformed into SDS (Tanner and Whitehouse, 1976). Mid-parental height SDS (MPH SDS) was calculated for each child, taking into consideration the sex of the child (Tanner et al., 1975). Children’s height SDS were then individually corrected for their genetic potential (parental contribution), using the formula: child’s height SDS minus MPH SDS. Children’s and parents’ BMI were transformed into SDS and mean parental BMISDS (MPBMISDS) was calculated for each child (Freeman et al., 1995). Each child’s corrected BMISDS was calculated individually using the formula: child’s BMISDS minus MPBMISDS. The SDS system expresses the extent of the deviation of a given value from the standard population mean. Each SDS is calculated as the individual value minus the mean value for the reference population (given gender and age), divided by the standard deviation of the reference population. Thus, the mean reference point is zero (de Onis and Habicht, 1996).

A fasting morning blood sample was obtained from each child for the assessment of metabolic and growth factors. Plasma insulin was measured using an Abbott AxSYM system (Abbott Laboratories, Abbott Park, IL, USA) by microparticle enzyme immunoassay (Abbott Diagnostics, Wiesbaden, Germany) with an inter-assay coefficient of variation (CV) of <5%. Glucose, triglyceride, cholesterol, HDL and LDL concentrations were measured on a Hitachi 902 autoanalyzer (Hitachi High Technologies, Madison, WI, USA). Each child had a bone age X-ray to assess biological age, based on birthweight data for ≏ 500 000 ‘healthy newborns’ in Sweden (Niklasson et al., 2001).
Corporation, Tokyo, Japan) by enzymatic colorimetric assay (Roche, Mannheim, Germany) with an inter-assay CV of 1.2% for glucose and <5% for cholesterol, triglycerides, HDL and LDL. Insulin resistance was calculated using the homeostasis model (HOMA-IR; Muniyappa et al., 2008). Commercially available ELISAs (R&D Systems, Minneapolis, MN, USA) were used to evaluate plasma IGF-1 (DSL-10-100, intra-assay CV 2.8%, inter-assay CV 9.2%) and IGF-binding protein 3 (IGFBP-3; DSL-10-6600, intra-assay CV 3.1%, inter-assay CV 9.9%). Commercially available ELISA kits (Meddiagnost, Reutlingen Germany) were used to evaluate IGFBP-2 (E-30, intra-assay CV 1.9%, inter-assay CV 6.3%).

Statistical analysis
ANOVA was used to compare baseline characteristics of parents and children in each group (OSA, subfertile and fertile). Data were analysed with both sexes combined, as well as separately for boys and girls. Linear mixed models followed by Tukey’s post hoc comparisons were used to investigate differences in anthropometric measures. Models included family as a random effect to control for the presence of siblings. Metabolic and endocrine data (plasma glucose, insulin, HOMA-IR, triglycerides, cholesterol, HDL, LDL, cholesterol: HDL ratio, IGF-1, IGF-2 and IGFBP-3) were analysed in a similar manner with child’s age and sex included in the model where appropriate. Multiple analysis of variance (MANOVA) was used to compare the combined effects on corrected height SDS, IGF-1 and IGFBP-3 among groups (OSA, subfertile and fertile). Means ± SEM are reported for baseline data; means ± SEM adjusted for other variables in the model are reported for other measures. Analyses were carried out in SAS v.9.1 (SAS Institute, Cary, NC, USA) and Minitab v.16 (Pennsylvania State University, PA, USA). Significance was determined if P < 0.05.

Ethics approval
Ethics approval for this study was provided by the Northern Y Regional Ethics Committee and written informed consent was obtained from parents of all participants. Depending on the child’s age, written or verbal consent was obtained from all children.

Results
Study population
Two hundred and six OSA mothers were eligible to be contacted for participation in the study when inclusion and exclusion criteria were applied. Ninety-two mothers (45%) were un-contactable due to out-of-date contact details and 30 mothers (15%) declined to participate. In all, 84 of 114 (74%) contactable candidates participated in the study. There were no discernable differences in known baseline characteristics between participants and non-participants.

A total of 352 children aged 7.33 ± 0.12 years were studied: 84 OSA subjects and 268 naturally conceived controls, comprising 54 children of subfertile parents and 214 children of fertile parents. The mothers of OSA children received drug treatment for ovarian stimulation with clomiphene alone (n = 26; 31%), a combination of FSH and clomiphene (n = 53; 63%) or FSH alone (n = 5; 6%), and most (n = 69; 82%) also underwent intratuterine insemination as part of treatment. 80% of OSA children (35 girls and 32 boys) had parents with unexplained infertility, while the remaining 20% (10 girls and 7 boys) had a father with mild male factor infertility (21%). Among the naturally conceived controls, 54 of 268 (20%) were children of subfertile parents, as they were conceived after 12 months or more of unprotected intercourse.

Parental and birth characteristics of each group are summarized in Table I. Boys and girls from each group (OSA, subfertile and fertile parents) were of similar age (Tables II and III), and their age and sex distribution was also similar (data not shown). Comparison of children of subfertile parents to children of fertile parents yielded no differences in the measured anthropometric, endocrine or metabolic parameters. OSA children were shorter than children of subfertile parents (SDS: −0.075 ± 0.09 versus 0.32 ± 0.07, P = 0.001) and were also shorter than children of fertile parents (−0.075 ± 0.09 versus 0.45 ± 0.10, P = 0.004) when corrected for MPHSDS. OSA children also had lower BMI SDS than children of subfertile parents (SDS: −0.90 ± 0.15 versus −0.37 ± 0.17; P = 0.06) and children of fertile parents (−0.90 ± 0.15 versus −0.34 ± 0.10; P = 0.008) when corrected for MPBMISDS.

When the sexes were compared separately, OSA boys were shorter than boys of subfertile (P = 0.03) and fertile (P = 0.01) parents after correction for MPHSDS (Table II). Similarly, OSA girls tended to be shorter than girls of subfertile (P = 0.06) but not shorter than girls of fertile parents (P = 0.17) when corrected for MPBMISDS (Table III). OSA boys had lower IGFBP-3 levels than boys of subfertile (P = 0.03) and fertile parents (P = 0.03; Table II). Comparison of the combined effects on corrected height SDS, IGF-1 and IGFBP-3 among groups demonstrated distinct differences between OSA boys compared with boys of subfertile (P = 0.002) and fertile parents (P = 0.003). It should also be noted that boys and girls in all groups displayed similar biological maturity based on individual bone age assessments (Tables II and III).

Overall, the OSA children had lower fasting glucose than the control children (children of subfertile and fertile parents combined; 4.62 ± 0.07 versus 4.81 ± 0.04 mmol/l; P = 0.006). In addition, HDL concentrations in OSA girls were higher than in girls of fertile parents (P = 0.01) and tended to be higher than in girls of subfertile parents.

| Table I Parental and birth characteristics of children in OSA, subfertile and fertile groups. |
|-----------------|-------|-------|-------|
| Parameter       | OSA   | Subfertile | Fertile |
| N               | 84    | 54     | 214    |
| Mid-parental height SDS | 0.95 ± 0.08 | 0.64 ± 0.08* | 0.86 ± 0.05 |
| Maternal BMI SDS | 0.30 ± 0.12 | 0.66 ± 0.15 | 0.34 ± 0.07 |
| Mean parental BMI SDS | 0.63 ± 0.09 | 0.93 ± 0.12 | 0.70 ± 0.05 |
| Maternal age at delivery (years) | 36.9 ± 0.4 | 35.5 ± 0.33* | 32.8 ± 0.31† |
| Time to pregnancy (mths) | 24 ± 2.3 | 22 ± 1.5 | 2.9 ± 0.15† |
| Gestation (wks) | 39.1 ± 0.15 | 39.3 ± 0.17 | 39.5 ± 0.08 |
| Birthweight SDS | 0.05 ± 0.1 | 0.14 ± 0.12 | 0.22 ± 0.06 |
| Firstborns (%) | 56    | 61     | 50     |
| Sex ratio (% males) | 47    | 46     | 47     |
| Breast-feeding rates at 6 months (%) | 62    | 59     | 63     |

BMI, body mass index; SDS, standard deviation score. Where appropriate, data are means ± SEM.
*P < 0.05 for comparison with OSA, †P < 0.05 for comparison with subfertile group.
metabolic and hormonal outcomes in OSA children born at term
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This study found that, when corrected for their genetic height poten-
tion of ovarian stimulation to the height of either OSA or IVF children
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is likely to persist into adulthood. Fur-
addition, ovarian stimulation for IVF differs from OSA, as it utilizes dif-
factor-binding protein; LDL, low-density lipoprotein; SDS, standard deviation score.
*P < 0.05 for comparison with OSA.

(P = 0.09; Table III). As a result, OSA girls had lower total cholesterol
to HDL ratios than girls of fertile parents (P = 0.01), and also tended
this way compared with girls of subfertile parents (P = 0.09; Table III).

**Discussion**

This study found that, when corrected for their genetic height poten-
tial, children conceived following OSA were shorter than children of fertile and subfertile parents. On average OSA boys were 3 cm shorter than boys of subfertile and fertile parents, with a trend
towards shorter stature in OSA girls compared with girls of subfertile
parents. Importantly, the children studied did not display evidence of
delayed biological maturation, as children’s bone ages matched their
chronological age (Tanner et al., 1975). Thus, the observed height re-
duction among OSA children is likely to persist into adulthood. Fur-
thermore, when the combined effects on corrected height SDS, IGF-1 and IGFBP-3 were evaluated, there were clear differences
between OSA boys and boys of fertile and subfertile parents.

To our knowledge, this is the first study to assess auxological, metabolic and hormonal outcomes in OSA children born at term
outcomes in fertility treatment children (OSA or IVF) to have two
separate control groups, consisting of children of subfertile and fertile parents. The finding of shorter stature in OSA children was un-
expected, and contrasts with a previous study that found similar
height to controls in OSA children born at very low birthweight
(Makhoul et al., 2009). Our findings also contrast with the taller
stature of IVF children found in several studies (Pruksananonda,
2001; Miles et al., 2007; Ceelen et al., 2009; Makhoul et al., 2009; Green et al., 2010).

IVF differs substantially to OSA, as IVF involves several further steps,
including external fertilization and embryo culture and selection. In
addition, ovarian stimulation for IVF differs from OSA, as it utilizes dif-
ferent drug regimens at higher doses, aiming to produce 6–10 follicles
for IVF versus 2–3 follicles for OSA (Van Rumste et al., 2008). Further-
more, clomiphene is frequently utilized in OSA, but rarely used in
ovarian stimulation for IVF (Berker et al., 2011). Hence, the contribu-
tion of ovarian stimulation to the height of either OSA or IVF children
is likely to be complex, and may depend on several factors including
the type and degree of ovarian stimulation.

One potential reason for the differences observed between OSA
and naturally conceived children may be that ovarian stimulation

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**Table II Anthropic and fasting serum endocrine and metabolic parameters in boys from OSA, subfertile and fertile
groups.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Boys</th>
<th>Subfertile</th>
<th>Fertile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>39</td>
<td>26</td>
<td>123</td>
</tr>
<tr>
<td>Anthropometric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>7.49 ± 0.37</td>
<td>7.56 ± 0.47</td>
<td>7.34 ± 0.19</td>
</tr>
<tr>
<td>Bone age–age (years)</td>
<td>−0.01 ± 0.13</td>
<td>−0.51 ± 0.22</td>
<td>−0.16 ± 0.10</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.74 ± 0.16</td>
<td>0.94 ± 0.19</td>
<td>0.95 ± 0.09</td>
</tr>
<tr>
<td>HtSDS–MPHSDS</td>
<td>−0.18 ± 0.14</td>
<td>0.42 ± 0.16*</td>
<td>0.35 ± 0.08*</td>
</tr>
<tr>
<td>Sitting HtSDS</td>
<td>0.02 ± 0.24</td>
<td>0.36 ± 0.30</td>
<td>0.53 ± 0.15</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.06 ± 0.17</td>
<td>−0.07 ± 0.21</td>
<td>−0.05 ± 0.10</td>
</tr>
<tr>
<td>BMI SDS–MPBMI SDS</td>
<td>−0.6 ± 0.22</td>
<td>−0.32 ± 0.27</td>
<td>−0.13 ± 0.14</td>
</tr>
<tr>
<td>Body fat %</td>
<td>16.1 ± 0.9</td>
<td>16.5 ± 1.5</td>
<td>14.8 ± 0.5</td>
</tr>
<tr>
<td>Endocrine and metabolic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (mU l⁻¹)</td>
<td>4.66 ± 0.37</td>
<td>5.34 ± 0.43</td>
<td>4.97 ± 0.22</td>
</tr>
<tr>
<td>Glucose (mmol l⁻¹)</td>
<td>4.64 ± 0.07</td>
<td>4.86 ± 0.08*</td>
<td>4.83 ± 0.04</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.97 ± 0.09</td>
<td>1.19 ± 0.10</td>
<td>1.10 ± 0.05</td>
</tr>
<tr>
<td>Cholesterol (mmol l⁻¹)</td>
<td>4.10 ± 0.13</td>
<td>4.15 ± 0.16</td>
<td>4.26 ± 0.08</td>
</tr>
<tr>
<td>HDL (mmol l⁻¹)</td>
<td>1.36 ± 0.06</td>
<td>1.33 ± 0.07</td>
<td>1.35 ± 0.03</td>
</tr>
<tr>
<td>Chol: HDL ratio</td>
<td>3.26 ± 1.90</td>
<td>3.65 ± 1.00</td>
<td>4.95 ± 1.40</td>
</tr>
<tr>
<td>LDL (mmol l⁻¹)</td>
<td>2.40 ± 0.10</td>
<td>2.45 ± 0.13</td>
<td>2.44 ± 0.06</td>
</tr>
<tr>
<td>Triglycerides (mmol l⁻¹)</td>
<td>0.68 ± 0.05</td>
<td>0.79 ± 0.06</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>IGF-1 (µg l⁻¹)</td>
<td>93 ± 6</td>
<td>111 ± 7</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>IGF-2 (µg l⁻¹)</td>
<td>763 ± 19</td>
<td>756 ± 19</td>
<td>733 ± 8</td>
</tr>
<tr>
<td>IGFBP-3 (µg l⁻¹)</td>
<td>2309 ± 126</td>
<td>2829 ± 161*</td>
<td>2683 ± 76*</td>
</tr>
</tbody>
</table>

Data are means ± SEM. BMI, body mass index; BMI SDS–MPBMI SDS, child’s BMI SDS corrected for mean parental BMI SDS; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HtSDS–MPHSDS, child’s height SDS corrected for mid-parental height SDS; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; LDL, low-density lipoprotein; SDS, standard deviation score.

*P < 0.05 for comparison with OSA.
leads to alterations in genomic imprinting in the oocyte or embryo, with consequent programming of endocrine changes. As the timing of imprinting overlaps with the timing of ovarian stimulation, interventions in oocyte maturation could have an impact on imprinting and methylation (Wilkins-Haug, 2009). In fact, several studies have found that ovarian stimulation for IVF alters DNA methylation of imprinted and non-imprinted genes (Sato et al., 2007; Gomes et al., 2009; Katari et al., 2009; Santos et al., 2010), and a recent investigation suggested that the degree of ovarian stimulation may influence the extent of methylation change in the oocyte (Market-Velker et al., 2010). Further, imprinted genes involved in growth regulation can also be altered by ovarian stimulation for IVF (Gomes et al., 2009). Although there is no direct evidence linking clomiphene to methylation changes, such alterations can be associated with tamoxifen (Badia et al., 2007; Feinberg, 2007), which is a structurally similar compound to clomiphene (Homburg, 2005). Thus, it is possible that ovarian stimulation may cause imprinting changes at critical points in early embryonic development, leading to the phenotypic differences seen in our study population. It is also conceivable that sexually dimorphic imprinting could explain the more pronounced height difference observed in OSA boys, but this remains speculative at this stage. Future work should examine the DNA of OSA children for differences in methylation of imprinted and non-imprinted genes when compared with naturally conceived children.

Another possible explanation for the observed differences in OSA children may be indirect effects of clomiphene citrate or FSH on the oocyte or developing embryo. FSH is used as part of ovarian stimulation for IVF, and this process has been shown to alter intrauterine chemokines, cytokines and growth factors; but it is unknown whether clomiphene has many well-recognized effects on the intrauterine environment, including altered embryonic adhesion (Valbuena et al., 1997), which may impact on embryonic development. Clomiphene is also associated with reduced serum levels of IGF-1 and increased levels of IGFBP-3 in some women after standard treatment for OSA, but it is unknown if these alterations affect the developing oocyte or embryo (Fiad et al., 1998; Shobokshi and Shaarawy, 2003). With metabolites of clomiphene detectable in maternal serum >1 month after administration (Homburg, 2005), it is possible that clomiphene could have

Table III  Anthropometric and fasting serum endocrine and metabolic parameters in girls from OSA, subfertile and fertile groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Girls</th>
<th>Subfertile</th>
<th>Fertile</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>45</td>
<td>28</td>
<td>91</td>
</tr>
<tr>
<td>Anthropometric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>7.10 ± 0.32</td>
<td>7.43 ± 0.47</td>
<td>7.24 ± 0.23</td>
</tr>
<tr>
<td>Bone age–age (year)</td>
<td>-0.26 ± 0.14</td>
<td>-0.09 ± 0.12</td>
<td>-0.11 ± 0.10</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.96 ± 0.14</td>
<td>0.68 ± 0.18</td>
<td>0.87 ± 0.10</td>
</tr>
<tr>
<td>HS–SDS–MPHSDS</td>
<td>0.00 ± 0.13</td>
<td>0.48 ± 0.15</td>
<td>0.29 ± 0.09</td>
</tr>
<tr>
<td>Sitting HS–SDS</td>
<td>0.22 ± 0.20</td>
<td>-0.07 ± 0.24</td>
<td>0.02 ± 0.14</td>
</tr>
<tr>
<td>BMISDS</td>
<td>-0.48 ± 0.15</td>
<td>-0.27 ± 0.18</td>
<td>-0.30 ± 0.10</td>
</tr>
<tr>
<td>BMISDS–MPBMISDS</td>
<td>-1.10 ± 0.18</td>
<td>-0.49 ± 0.22</td>
<td>-0.40 ± 0.13 *</td>
</tr>
<tr>
<td>Body fat %</td>
<td>18.9 ± 0.0</td>
<td>21.6 ± 1.2</td>
<td>19.1 ± 0.6</td>
</tr>
<tr>
<td>Endocrine &amp; metabolic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (mU L⁻¹)</td>
<td>5.30 ± 0.53</td>
<td>6.11 ± 0.66</td>
<td>6.33 ± 0.34</td>
</tr>
<tr>
<td>Glucose (mmol L⁻¹)</td>
<td>4.59 ± 0.07</td>
<td>4.67 ± 0.08</td>
<td>4.82 ± 0.04 *</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.10 ± 0.13</td>
<td>1.36 ± 0.15</td>
<td>1.40 ± 0.08</td>
</tr>
<tr>
<td>Cholesterol (mmol L⁻¹)</td>
<td>4.36 ± 0.14</td>
<td>4.44 ± 0.15</td>
<td>4.43 ± 0.09</td>
</tr>
<tr>
<td>HDL (mmol L⁻¹)</td>
<td>1.50 ± 0.05</td>
<td>1.33 ± 0.06</td>
<td>1.31 ± 0.03 **</td>
</tr>
<tr>
<td>Chol: HDL ratio</td>
<td>2.94 ± 0.15</td>
<td>3.40 ± 0.17</td>
<td>3.51 ± 0.10 **</td>
</tr>
<tr>
<td>LDL (mmol L⁻¹)</td>
<td>2.49 ± 0.11</td>
<td>2.60 ± 0.12</td>
<td>2.66 ± 0.07</td>
</tr>
<tr>
<td>Triglycerides (mmol L⁻¹)</td>
<td>0.77 ± 0.05</td>
<td>0.77 ± 0.06</td>
<td>0.81 ± 0.03</td>
</tr>
<tr>
<td>IGF-1 (μg L⁻¹)</td>
<td>123 ± 9</td>
<td>116 ± 10</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>IGF-2 (μg L⁻¹)</td>
<td>795 ± 15</td>
<td>755 ± 17</td>
<td>753 ± 9</td>
</tr>
<tr>
<td>IGFBP-3 (μg L⁻¹)</td>
<td>2786 ± 45</td>
<td>2647 ± 148</td>
<td>2905 ± 92</td>
</tr>
</tbody>
</table>

Data are means ± SEM. BMI, body mass index; BMISDS–MPBMISDS, child’s BMISDS corrected for mean parental BMISDS; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HS–SDS, height SDS; HS–SDS–MPHSDS, child’s height SDS corrected for mid-parental height SDS; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; LDL, low-density lipoprotein; SDS, standard deviation score.

*P < 0.05, **P < 0.01 for comparison with OSA.
subtle effects in the developing oocyte or embryo, leading to subsequent phenotypic changes.

This study compared outcomes in OSA children to those born of subfertile and fertile parents in order to differentiate the contribution of parental subfertility separately from that of the fertility treatment itself. It is therefore of interest that children of fertile and subfertile parents were similar in all anthropometric, endocrine and metabolic parameters measured in our study, even though the subfertile control group was comparatively smaller ($n = 54$). The inclusion of children of subfertile parents in this study was important, as they are the most logical comparison group for children conceived by OSA or IVF (Pandian et al., 2005). It seems that parental subfertility is an important factor determining peri-natal and childhood outcomes in IVF offspring (Romundstad et al., 2011), and may account in part for the higher rate of congenital abnormalities and imprinting disorders amongst the IVF population (Ludwig et al., 2005; Zhu et al., 2006; Doornbos et al., 2007; Manipalviratn et al., 2009; Källen et al., 2010). However, as children of fertile and subfertile parents had similar heights, the observed shorter stature among OSA children in our study is unlikely to be explained by parental subfertility. Nonetheless, it is important to emphasize that OSA and IVF children are in reality a distinct population to children of subfertile parents, as the latter were eventually conceived naturally, whereas OSA and IVF children were not.

We also observed metabolic differences (including lower fasting glucose and higher HDL) in OSA children. To our knowledge, no previous studies have examined metabolism in OSA children, and those on IVF children yielded conflicting results (reviewed by (Kanaka-Gantenbein et al., 2010)). It is unclear whether these relatively subtle metabolic changes could have any long-term significance, but they are further evidence that OSA children are different from those conceived naturally.

The strengths of this study include restriction of the study population to a higher socio-economic group and single ethnicity in an effort to reduce confounders. Further, the height of each child in our study was individually corrected for parental stature (MPHSDS), which is the most important determinant of childhood height (Tanner et al., 1975). We encountered only three previous studies that corrected for this fundamental factor of genetic height when assessing childhood height in OSA or IVF children (Miles et al., 2007; Makhoul et al., 2009; Green et al., 2010). In contrast, a possible limitation of our study is that we cannot rule out with certainty the existence of PCOS cases among the mothers of OSA children or control groups. However, any OSA mothers with a positive ultrasound or history of PCOS were excluded from the study, and all control mothers had a negative history of PCOS (Dewailly et al., 2011). Furthermore, it is worth noting that no mothers (OSA and controls) had a history of glucose intolerance, and the mean maternal BMI SDS was well within the normal BMI SDS range in all groups. Another possible weakness of the study is that the duration to conception reported by parents in the subfertile group may be subject to recall bias, but we optimized our methods to maximize recall accuracy. A further possible weakness was the relatively small number of children of subfertile parents ($n = 54$) compared with the number of children of fertile parents ($n = 214$). Therefore, a larger study with a greater number of children in the subfertile group may be required to clarify possible differences in anthropometric, metabolic or hormonal outcomes between children of fertile and subfertile parents.

**Conclusion**

OSA children, particularly boys, have shorter stature than children of fertile and subfertile parents. There is also evidence that OSA children have an altered metabolic phenotype. Since OSA children comprise up to 7% of all births in Western countries, it is important to determine whether these differences persist or accentuate during puberty and into adulthood. Further studies are necessary to fully understand any potential long-term consequences to children and adults conceived with this widely used fertility treatment.

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**Authors’ roles**

W.S.C., P.L.H., T.S., and J.C.P. conceived and designed the study. T.S. collected the data, which were analysed by E.M.R., T.S., M.P.G., H.L.M. and F.M. interpreted the data. T.S. wrote the initial drafts of the paper. W.S.C., P.L.H., T.S., J.C.P., F.M., E.M.R., M.P.G. and H.L.M. revised the paper. All authors approved the final version.

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**Conflict of interest**

T.S., E.L.R., M.P.G., H.L.M., F.M., P.L.H. and W.S.C. have nothing to declare. J.C.P. is employed as the scientific director for Fertility Associates. None of the authors have a competing interest to declare.

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