Serum inhibin B concentrations in pubertal boys conceived by ICSI: first results

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BACKGROUND: Currently, no published data exist about the gonadal function of children born after ICSI. To evaluate potential risk of testicular seminal dysfunction in boys born to fathers with compromised spermatogenesis, serum inhibin B (as a marker for spermatogenesis) was assessed.

METHODS: We recruited 50 pubertal adolescents from the oldest cohort of infants born following ICSI. Cross-sectional serum inhibin B levels of all 50 ICSI adolescents, and longitudinal serum inhibin B (assessed at 8 and 14 years) in 25 boys, are reported.

RESULTS: A statistically significant increase in inhibin B levels was observed between 8 (mean 69 ng/l, SD ± 35) and 14 years (mean 145 ng/l, SD ± 41; P = 0.001). In three quarters of the ICSI boys an increase in serum inhibin B levels of at least 30% between 8 and 14 years was observed. In all but 4 of the 14-year-old ICSI boys serum inhibin B was normal. Serum inhibin B levels in boys from fathers with severe oligozoospermia did not differ from concentrations in boys from fathers without severe oligozoospermia (154 ± 51 and 142 ± 47 ng/l, respectively; P = 0.4).

CONCLUSIONS: The majority of ICSI boys have a significant increase in serum inhibin B, attaining normal values for pubertal status at the age of 14 years. ICSI adolescents from fathers with severely compromised spermatogenesis do not have lower inhibin B levels than those with fathers with normal spermatograms. Further follow-up of the spermatogenic potential of ICSI teenagers up to young adulthood is mandatory to confirm a normal reproductive capacity.

Key words: intracytoplasmic sperm injection / inhibin B / testis / follow-up / puberty

Introduction

With the introduction of ICSI, the prognosis for couples with severe male-factor infertility has dramatically improved (Palermo et al., 1992; Van Steirteghem et al., 1996). On the other hand, since genetic factors play an important role in the pathogenesis of male-factor infertility, children conceived by ICSI may be at risk for later infertility problems (Kurinczuk, 2003). Y chromosome deletions involving spermatogenic genes are the most frequently recognized genetic cause of male infertility (Silber and Repping, 2002). Vertical transmission of microdeletions of the Y chromosome through the use of ICSI has been reported (Page et al., 1999; Cram et al., 2000). In up to 15% of male-factor infertility cases, male infertility is associated with endocrine conditions such as gonadotrophin deficiency, primary testicular failure and androgen resistance (Bhasin, 2007). Indeed, adequate functioning of the male reproductive hypothalamic-pituitary-gonadal axis depends on feedback systems between several hormones (Kuijper et al., 2007). Pituitary-released FSH stimulates testicular Sertoli cells to produce inhibin B. Inhibin B, a glycoprotein, is low during the pre-pubertal period, but shows a rapid rise in early adolescence (Andersson et al., 1997; Crofton et al., 2002). The rise in inhibin B converges with spermarche (Nielson et al., 1986). Moreover, inhibin B is known as a marker for spermatogenesis in adult men (Jensen et al., 1997; Pierik et al., 1998). Serum inhibin B levels are reduced in men with severely disturbed spermatogenesis (Anawalt et al., 1996; Klingmuller and Haidl, 1997).

The eldest ICSI offspring are now reaching adolescence. To date, no data exist on the reproductive function of ICSI-conceived boys beyond childhood.
To investigate if ICSI-conceived boys from fathers with compromised spermatogenesis are at risk for testicular germinal dysfunction, the change of inhibin B from pre-puberty to puberty and the relation of inhibin B to paternal sperm characteristics were evaluated in 14-year-old ICSI male adolescents.

Subjects and methods

From a prospective cohort study concerning cardiometabolic outcome of ICSI teenagers, involving a total of 59 boys and 59 girls, data from 50 boys reaching the age of 14 years between January 2008 and November 2009, were analysed. Nine out of the 59 boys refused blood sampling, but agreed to a clinical examination. In 25 ICSI boys, longitudinal data at 8 years (range: 8.0–9.0 years) (De Schepper et al., 2009) and 14 years (range: 13.6–15.1 years) are available. All included boys were singleton, Caucasian and born after at least 32 weeks’ gestation. With the exception of a surgical intervention for hypospadias (n = 1), orchidopexy (n = 1) and an urethral membrane resection (n = 1), no major genital surgery had been performed.

ICSI was performed using ejaculated sperm. Father’s spermogram (according to WHO guidelines 1999) showed the following: mean (and ranges) of the analysed volume was 3.8 (0.8–8.0) ml with a mean sperm concentration of 7.4 × 10⁶ (0.03 × 10⁶–52.0 × 10⁶)/ml and a mean motility count of 52.2 (0–100)%. In 32 fathers, total motile sperm count was below 5 × 10⁶/ml, representing severe oligozoospermia. All tested fathers (n = 44) had a normal karyotype.

A standard clinical examination including height and weight and scoring of pubertal development using Tanner staging was performed. Blood samples were taken between noon and 4 p.m. and were centrifuged within 1 h of collection. Sera were frozen at −C until analysis. Inhibin B levels were measured using a commercial ELISA (Inhibin B, Oxford Bio-Innovation Ltd., Oxford, UK). Reference ranges according to Andersson et al. (1997) were used. Technical aspects are described previously (De Schepper et al., 2009).

All parents gave written informed consent for clinical and hormonal testing. Data are expressed as mean (SD) or median. Ranges or 5th–95th percentiles are given where appropriate. Statistical analyses used were Student’s t-test and Kruskall–Wallis test for comparisons and Spearman’s rank order correlation test for correlation. Paired t-test was used for comparing inhibin B levels between 8 and 14 years. All analyses were performed using Statistical Package for the Social Sciences 16.0. A difference was considered significant when the two-tailed P < 0.05.

Results

At 14 years, hormonal data of 50 ICSI boys were assessed. Inhibin B levels are reported in Table I. Mean inhibin B levels did not differ across the four pubertal subgroups (P = 0.2). In 4 out of 50 ICSI adolescents, abnormally low inhibin B values for their pubertal status were found (Table I). In two boys, inhibin B levels were low-normal at 8 years and in two other boys no previous measurements were available.

In the 25 boys with longitudinal hormonal data, the mean inhibin B levels increased significantly from 69 (+ 35) ng/l (range: 9–139; median: 68 ng/l) at 8 years to 145 (+ 41) ng/l (range: 90–240; median: 135 ng/l) at the age of 14 years (P < 0.001; Fig. 1). In more than three quarters of the subjects, an increase of 30% of inhibin B levels was found, and in half the subjects inhibin B levels doubled. No difference in inhibin B level, age and pubertal maturity was found between the ICSI children with measurements at 8 and 14 years (n = 25) and those with only measurements at 14 years (n = 25; data not shown).

At 14 years, inhibin B levels did not correlate with levels at 8 years (P = 0.6) or paternal sperm parameters (concentration: P = 0.9; motility: P = 0.3). Boys born from men with severe oligozoospermia (n = 32) had comparable mean inhibin B values to those born from men with sperm concentrations above 5 × 10⁶/ml (154 ± 51 and 142 ± 47 ng/l, respectively; P = 0.4). Of the four boys with abnormally low inhibin B values according to their pubertal stage, two fathers had severe oligozoospermia (Table I).

<table>
<thead>
<tr>
<th>Table I Inhibin B levels in 14-year-old ICSI children (n = 50).</th>
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<tr>
<td><strong>Pubertal stage</strong></td>
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<tr>
<td>Inhibin B (ng/l)</td>
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<tr>
<td>Median</td>
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<td>Mean</td>
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<td>Reference dataa converted to ng/l</td>
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<td>Median</td>
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<td>N of boys with results below the 5th percentile compared with the reference group</td>
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<td>Actual inhibin B value (ng/l)</td>
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aAndersson et al. (1997).

bBoys born from fathers with severe oligozoospermia.

Figure 1 Change of inhibin B levels between 8 and 14 years in 25 ICSI boys.
**Discussion**

Data on the testicular function in boys born after ICSI are scarce. Mau Kai et al. (2007) reported that at the age of 3 months, serum levels of inhibin B were not different in ICSI from control boys. We confirmed that the majority of 8-year-old ICSI-conceived boys had normal serum inhibin B concentrations (De Schepper et al., 2009). In the present study, for the first time, longitudinal data on testicular function of pubertal boys conceived after ICSI are described, showing an increase in serum inhibin B levels from pre-puberty to puberty in all but one subject. It has previously been shown that inhibin B levels are low between 6 and 10 years, but rapidly increase after onset of puberty (Andersson et al., 1997; Crofton et al., 2002; Radicioni et al., 2005). Previous histological studies have shown that pubertal inhibin B production is dependent on the presence of germ cells (Andersson et al., 1997; Anderson and Sharpe, 2000). It is still a matter of debate when the highest increase in inhibin B levels occurs, between pubertal Stages 1 and 2 (Andersson et al., 1997), between Stages 2 and 3 (Crofton et al., 2002) or between Stages 3 and 4 (Radicioni et al., 2005). In this study, we did not find a difference in inhibin B levels across pubertal Stages G2, G3, G4 and G5, probably due to low number of children included in each subgroup. Nevertheless, ICSI children in our study showed a similar increase in median inhibin B levels between pre-puberty and pubertal Stage 3 (68–159 ng/l) compared with reference data (78–163 ng/l) (Andersson et al., 1997).

In this study, 4/50 (8%) of ICSI adolescents had serum inhibin B measurements that were below the lower limits for their pubertal stage. Since the normal range for laboratory tests is usually defined by the 95% confidence interval, it can be expected that 2.5% of the individuals have values below the lower limit. We recognize that, unfortunately, no locally developed reference values are available for comparison with the described limited study group. Furthermore, we agree that care should be taken in assessing the spermatogenic potential by the use of only one serum inhibin B measurement in male adolescents because of the known intra-individual variation and the limited diagnostic efficiency of inhibin B testing observed in adults with idiopathic infertility and the lack of longitudinal serum inhibin B data in adolescents in predicting a disturbed spermatogenesis (Andersson et al., 2004).

In accordance with results from our previous study at 8 years, we did not find lower mean inhibin B levels at 14 years in boys from fathers with severe oligozoospermia. However, the number of cases in this report is limited and the period of evaluation is too short to draw the conclusion that boys of fathers with compromised spermatogenesis have normal spermatogenic functions in adulthood.

In conclusion, inhibin B levels are generally within normal ranges and display a physiological peri-pubertal rise in ICSI boys between 8 and 14 years. Boys from fathers with severely compromised spermatogenesis did not have lower inhibin B levels, neither during childhood, nor at mid-puberty compared with sons of fathers with less compromised spermatogenesis. Further follow-up of the spermatogenic potential of ICSI adolescents is mandatory to confirm the perspective of a normal reproductive capacity.

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