Serum inhibin B determination is predictive of successful testicular sperm extraction in men with non-obstructive azoospermia

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Recent work indicates that serum inhibin B is a useful marker of spermatogenesis and inhibin B production sufficient to maintain detectable serum concentrations in adults depends on spermatogenic activity. The purpose of the present study was to investigate the usefulness of serum inhibin B measurement to predict the success of testicular sperm extraction (TESE) in 17 men with nonobstructive azoospermia to be treated by intracytoplasmic sperm injection (ICSI) (group 1). Two additional groups were used as positive controls; group 2 comprised 22 infertile men having obstructive azoospermia, and group 3, which included 29 semen donors having normal seminal parameters. Follicle stimulating hormone (FSH) was significantly higher (P < 0.01) and inhibin B significantly lower (P < 0.001), in group 1 as compared with groups 2 and 3. Serum inhibin B concentrations were significantly higher (P < 0.001) among successful TESE men as compared with those having failed TESE. In contrast, no differences were detected between these two groups with respect to serum FSH or testicular size. In addition, serum inhibin B but not FSH discriminated between successful and failed TESE in group 1 subjects as compared with control groups. According to the receiver operating characteristics curve analysis, the best inhibin B value for discriminating between successful and failed TESE was >40 pg/ml (sensitivity 90%, specificity 100%). It is concluded that inhibin B measurement is a useful non-invasive predictor of spermatogenesis and thus, all azoospermic males should have serum inhibin B concentrations determined in addition to FSH measurement and karyotyping prior to undergoing TESE.

Key words: ICSI/inhibin B/male infertility/non-obstructive azoospermia/TESE

Introduction

Recent progress in assisted reproduction techniques has made it possible to obtain spermatozoa surgically in azoospermic men whose condition is either due to primary gonadal failure or vas deferens obstruction (congenital or acquired) (Cha et al., 1997). However, these new techniques imply the use of testicular biopsy which is an invasive procedure that may be associated with potential complications (Schlegel and Su, 1997). With this in mind, different conventional markers of positive testicular sperm extraction (TESE) in azoospermic men have been recently investigated. In obstructive azoospermia, testicular size, plasma FSH and testicular histology have proven to be useful (Mulhall et al., 1997; Ezeh et al., 1998). However, no definitive marker has been described in cases of secretory azoospermia. Thus, it has been stressed that men should not be excluded from TESE based on serum FSH concentration, age, prior histopathological pattern or cytologic/wet preparation results (Martin-du-Pan and Bischof, 1995; Mulhall et al., 1997; Ezeh et al., 1998; Gil-Salom et al., 1998). Recent reports have shown that serum inhibin B is a useful marker of spermatogenesis (Anawalt et al., 1996; Ezeh et al., 1998; Pierik et al., 1998) and inhibin B production sufficient to maintain detectable serum concentrations in adults depends on spermatogenic activity (Petersen et al., 1999). The aim of the present study was to investigate the value of serum inhibin B measurement as a predictor of the success of TESE in men with non-obstructive azoospermia to be treated by intracytoplasmic sperm injection (ICSI).

Materials and methods

Subjects

A total of 17 consecutive men who presented with clinical and laboratory data indicating non-obstructive azoospermia underwent TESE in order to perform ICSI (group 1 or study group). Mean male age was 32.3 ± 0.6 years (mean ± SEM) (range 29–36). Subjects underwent a full clinical evaluation and the developmental, social, family, medical and reproductive histories as well as the history of urological operations were documented in all men. Each patient underwent physical examination including examination of vasa deferens, epididymes, and testicular size estimated with Prader orchidometer. Both karyotype and the DAZ gene were normal in all men in the study group. Twelve of the men were diagnosed as having idiopathic non-obstructive azoospermia, three had maldescended testis and two had a history of chemotherapy. All couples were given full and complete information about the ICSI technique with TESE. Local ethical committee approval was obtained prior to the introduction of any assisted reproduction techniques used in this study. Informed consent was obtained from all couples. Two additional groups were used as positive controls. Group 2 comprised 22 infertile men having obstructive azoospermia (absence of vasa deferens, n = 10; vasectomy, n = 7; previous genital inflammation, n = 5), and group 3 included 29 men having normal...
Seminal parameters and who were donors from the semen bank of our assisted reproduction unit.

**Seminal study**

Semen samples were produced by masturbation after 3–6 days of sexual abstinence and collected into sterile containers. The azoospermia was confirmed by at least two seminal analyses (>4 weeks apart) which were carried out as described in the World Health Organization Manual (WHO, 1999).

**Hormone analyses**

Hormones were measured using commercially available kits. FSH serum concentrations were measured by an immunoenzymatic assay with two monoclonal antibodies (Immuno 1; Technicon, Bayer, Tarrytown, NY, USA) and data were expressed in terms of IRP 78/549. The sensitivity of the assay was 0.1 IU/l and the inter-assay coefficient of variation was 2.7%. Dimeric inhibin B was measured by a solid-phase sandwich enzyme-linked immunosorbent assay which used two monoclonal antibodies (Serotec, Oxford, UK). The first monoclonal antibody is specific for the βB subunit of inhibin; the second one was directed to the α-subunit and coupled to alkaline phosphatase. The sensitivity of the assay was 15 pg/ml and the intra-assay and inter-assay coefficients of variation were <11 and 15% respectively.

**TESE and histopathology**

Testicular biopsies and sperm extraction were not always synchronized with the day of oocyte retrieval and the ICSI procedure. The testicular sperm retrievals were performed from bilateral testicular biopsies attained between the two study groups (successful and unsuccessful with the day of oocyte retrieval and the ICSI procedure. The testicular Whitney U-test and the non-parametric ANOVA test. The discrimination assay and inter-assay coefficients of variation were characteristics (ROC) analysis (Hanley and McNeil, 1982; Zweig and Campbell, 1993). Sensitivity, specificity, diagnostic accuracy and the area under the ROC curve (AUC ROC) were obtained for each model, 95% confidence intervals were calculated for each of the estimates. Areas of 1.0 and 0.5 denote no overlapping and no discrimination respectively between groups. As the data were non-normally distributed results are presented as mean ± SEM (and range) rather than mean ± SD.

**Results**

All testicular biopsies were considered suitable for histopathological analysis. The relative frequencies of the testicular histological patterns were focal spermatogenesis in seven subjects (41%), focal maturation arrest in seven (41%), Sertoli cell-only pattern in two (12%), and tubular sclerosis in one (6%). None of the subjects had hypospermatogenesis as an isolated abnormality.

**Ovarian stimulation and ICSI**

Ovarian stimulation was carried out with FSH under pituitary suppression with gonadotrophin-releasing hormone (GnRH) agonist according to a protocol previously reported (Balasch et al., 1996). In all women, pituitary desensitization was achieved by s.c. administration of leuprolide acetate (Procrin; Abbott Laboratories, Madrid, Spain) (1 mg daily, which was reduced to 0.5 mg after ovarian arrest was confirmed) started in the midluteal phase of the previous cycle. Gonadotrophin stimulation of the ovaries was started when serum oestradiol concentrations declined to <50 pg/ml and a vaginal ultrasonographic scan showed an absence of follicles >10 mm diameter. On days 1 and 2 of ovarian stimulation, 6 ampoules/day of highly purified FSH (Neo-Fertinorm, Serono SA, Madrid, Spain) were administered s.c. On days 3 to 7 of ovarian stimulation, 2 ampoules/day of FSH were administered to each patient. From day 8 onward, FSH was administered on an individual basis according to the ovarian response. The criteria for HCG administration were the presence of two or more follicles >18 mm in diameter in association with a consistent rise in serum oestradiol concentration. Oocyte aspiration was performed with vaginal ultrasonography 35–36 h after HCG administration. ICSI procedure was performed according to the method previously described (Palermo et al., 1992). Donor semen was used in those couples undergoing TESE, synchronized with the day of oocyte retrieval and having failed sperm recovery.

**Statistics and probability testing**

Data were analysed by SPSS statistical software using the Mann-Whitney U-test and the non-parametric ANOVA test. The discrimination attained between the two study groups (successful and unsuccessful sperm recovery) was evaluated with receiver-operating characteristic (ROC) analysis (Hanley and McNeil, 1982; Zweig and Campbell, 1993). Sensitivity, specificity, diagnostic accuracy and the area under the ROC curve (AUC ROC) were obtained for each model, 95% confidence intervals were calculated for each of the estimates. Areas of 1.0 and 0.5 denote no overlapping and no discrimination respectively between groups. As the data were non-normally distributed results are presented as mean ± SEM (and range) rather than mean ± SD.
Table I. FSH and inhibin serum concentrations in the three groups studied and in group 1 patients with successful and failed sperm retrieval

<table>
<thead>
<tr>
<th>Groups</th>
<th>FSH (IU/l)</th>
<th>Inhibin B (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 17)</td>
<td>12.7 ± 1.1 (6.2–21.4)</td>
<td>57.0 ± 8.8 (19–129)</td>
</tr>
<tr>
<td>TESE+ (n = 10)</td>
<td>12.4 ± 1.3 (6.2–18.3)</td>
<td>78.3 ± 10.5 (35–129)</td>
</tr>
<tr>
<td>TESE– (n = 7)</td>
<td>13.1 ± 2.2 (6.2–21.4)</td>
<td>26.7 ± 3.0 (19–40)</td>
</tr>
<tr>
<td>2 (n = 22)</td>
<td>4.5 ± 0.5 (1–12)</td>
<td>118 ± 9.7 (60–200)</td>
</tr>
<tr>
<td>3 (n = 29)</td>
<td>4.1 ± 0.3 (2–8.9)</td>
<td>117.6 ± 7.1 (50–185)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (range).

*Group 1 = non-obstructive azoospermia; group 2 = obstructive azoospermia; group 3 = semen donors; TESE+ = successful sperm recovery; TESE– = failed sperm recovery.

Table II. Statistical comparisons between groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups compared</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>FSH</td>
<td>1 versus 2 or 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>TESE+ versus TESE–</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TESE+ versus 2 or 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>TESE– versus 2 or 3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>2 versus 3</td>
<td>NS</td>
</tr>
<tr>
<td>Inhibin B</td>
<td>1 versus 2 or 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>TESE+ versus TESE–</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>TESE+ versus 2 or 3</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>TESE– versus 2 or 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>2 versus 3</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS= not significant

*Group 1 = non-obstructive azoospermia; group 2 = obstructive azoospermia; group 3 = semen donors; TESE+ = successful sperm recovery; TESE– = failed sperm recovery.

successful and failed sperm retrieval. Table II summarizes the statistical comparisons between groups. FSH was significantly higher (P < 0.001) and inhibin B significantly lower (P < 0.001), in group 1 as compared with groups 2 and 3. The relationship of endocrine profile to TESE indicated that serum inhibin B but not FSH concentrations were significantly higher among successful TESE subjects as compared with those having failed TESE. No differences were detected between these two groups with respect to testicular volume (7.9 ± 0.57 ml and 8 ± 1.5 ml for TESE+ and TESE– groups respectively). In addition, serum inhibin B but not FSH discriminated between successful and failed TESE in group 1 subjects as compared with control groups (Tables I and II). As expected, no differences were found between control groups 2 and 3 with respect to hormone concentrations.

To analyse further the diagnostic accuracy (predictive value of sperm retrieval) of inhibin B to discriminate between success and failure with TESE, the AUCROC curves determined with ROC analysis for serum FSH and inhibin B as well as testicular size are also shown (Figure 1 and Table III). The AUCROC for inhibin B in predicting the likelihood of success with TESE was significantly higher than those for FSH and testicular size. The ROC curve analysis was also used to determine the best threshold values for FSH, inhibin B and testicular size in predicting success or failure with TESE. The best inhibin B value for discriminating between successful and failed TESE was >40 pg/ml (sensitivity 90%, specificity 100%).

Discussion

In couples with infertility secondary to non-obstructive azoospermia, no corrective treatment is available except in cases of hypogonadotrophic hypogonadism. Recovery of testicular spermatozoa from azoospermic men for ICSI is a recent major advance in the treatment of such couples. Births of children conceived with testicular spermatozoa from men with deficient spermatogenesis leading to secretory azoospermia have been reported (Tournaye et al., 1995; Cha et al., 1997). Thus, TESE combined with ICSI offers azoospermic men the possibility of fathering their own genetic children even if they do not reveal normal spermatogenesis. As a result many infertile couples who previously had donor spermatozoa insemination as the only therapeutic alternative are at present, being offered this novel treatment.

ICSI using testicular spermatozoa is certainly a valid treatment option (Tournaye et al., 1995, Cha et al., 1997; Gil-Salom et al., 1998). TESE, however, may not always be successful in all azoospermic men (Martin-du-Pan and Bischof, 1995; Tournaye et al., 1995, 1997; Mulhall et al., 1997; Ezeh et al., 1998; Gil-Salom et al., 1998). ICSI using TESE from azoospermic men involves treatment for both partners as the husband undergoes surgery for testicular sperm recovery and the woman undergoes ovarian stimulation for oocyte retrieval. Therefore, an unsuccessful sperm recovery procedure has important emotional and financial implications which emphasise the importance of determining those factors predictive of a successful TESE. Objective counselling based on such predictive factors could offer realistic expectations for both the couple and the physician (Tournaye et al., 1997).

Recent reports have suggested that recovery of testicular spermatozoa may be possible in >50% of cases of true ‘non-obstructive’ azoospermia regardless of clinical parameters concerning size of testes or plasma FSH concentrations (Cha et al., 1997; Tournaye et al., 1997; Ezeh et al., 1998) a fact which is in agreement with results in the present study. In
fact, the increasing use of testicular screening has clearly demonstrated that plasma FSH can no longer be used as a guide for selection of azoospermic men for trials of TESE in assisted reproduction (Chen et al., 1996; Ezeh et al., 1998). Men with non-obstructive azoospermia may have areas of preserved spermatogenesis in the testicles but despite that, some clinical and histopathological parameters are associated with significantly different sperm recovery rates and it is not possible to predict with certainty the outcome of TESE in an individual patient. Therefore, the presence of spermatozoa in a single testicular biopsy does not guarantee a complete lack of spermatozoa in the testes and repetitive multiple biopsies may enable recovery of sufficient spermatozoa for microinjection despite a negative preliminary biopsy, suggesting focal hypoplasmatogenesis (Gottschalk-Sabag and Weiss, 1995; Tournaye et al., 1997). However, ICSI requires tight co-ordination with treatment of the woman, and repetitive surgery has of spermatogenesis and thus, all azoospermic males should be evaluated (Tournaye et al., 1997). In conclusion, inhibin B is a useful non-invasive predictor of spermatogenesis and thus, all azoospermic males should have serum inhibin B concentrations determined, in addition to FSH measurement and karyotyping, prior to undergoing TESE.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUCROC (95% CI)</th>
<th>Threshold</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Diagnostic accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/l)</td>
<td>0.52 (0.27–0.76)</td>
<td>≤17</td>
<td>90</td>
<td>42.8</td>
<td>70.5</td>
</tr>
<tr>
<td>Testicular volume (ml)</td>
<td>0.42 (0.19–0.68)</td>
<td>≤10</td>
<td>100</td>
<td>28.5</td>
<td>70.5</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>0.98 (0.8–1)</td>
<td>&gt;40</td>
<td>90</td>
<td>100</td>
<td>94.1</td>
</tr>
</tbody>
</table>

Values with different superscripts were significantly different (P < 0.01).

Acknowledgements
The authors thank Mrs Rosa Abellana and Mrs Esperanza González for their technical assistance.

References

Table III. Diagnostic accuracy of serum FSH and inhibin B concentrations, and testicular size to discriminate between successful and failed sperm retrieval
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Received on January 27, 2000; accepted on May 2, 2000