The significance of anti-Müllerian hormone concentration in seminal plasma for spermatogenesis

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BACKGROUND: The function of anti-Müllerian hormone (AMH) in seminal plasma in adulthood is uncertain. We examined the significance of seminal AMH for spermatogenesis. METHODS: We measured seminal concentrations of AMH in 39 oligozoospermic men (mean age ± SD, 32.7 ± 4.3 years) and 10 normal volunteers to examine the association of seminal AMH with spermatogenesis. The seminal concentrations of AMH in oligozoospermic men (149.3 ± 254.0 pmol/l) were significantly lower than in normal men (249.0 ± 167.7 pmol/l; P = 0.0337). Seminal AMH concentration correlated significantly with sperm concentration (r = 0.339, P = 0.0350) and mean testicular volume (r = 0.440, P = 0.246). The serum concentration of LH (r = −0.365, P = 0.0241), but not FSH, testosterone or estradiol, correlated significantly with AMH concentration in seminal plasma. CONCLUSIONS: AMH in seminal plasma may be important for sperm production, and is a good marker for Sertoli cell development.

Key words: anti-Müllerian hormone/oligozoospermia/Sertoli cell development/spermatogenesis

Introduction

Anti-Müllerian hormone (AMH) belongs to a superfamily of dimeric glycoproteins that are structurally similar to transforming growth factor-β, activins and inhibins (Massague, 1990). AMH is secreted bi-directionally by Sertoli cells: apically into the seminiferous tubules and basally toward the interstitium and the circulation. Blood concentrations of AMH are maintained at a high level until puberty, when they decrease dramatically (Josso et al., 1990), remaining very low in adulthood (Lee et al., 1996). Although the function of AMH in post-natal life is incompletely understood, AMH has been reported to control Leydig cell proliferation and steroidogenesis (Racine et al., 1998) and may also be related to germ cell differentiation (Cazorla et al., 1998). The data comparing seminal and serum AMH concentrations in adults suggest that after puberty AMH is secreted preferentially by the apical pole of the Sertoli cell toward the lumen of the seminiferous tubules, resulting in higher concentrations of AMH in the seminal plasma than in the serum (Fenichel et al., 1999). In addition, these authors suggested that seminal AMH might represent a marker that can be assessed non-invasively for the state of spermatogenesis in cases of non-obstructive azoospermia, and observed that very low seminal AMH concentrations were associated with spermatogenic failure (Fenichel et al., 1999). These observations suggest a close link between seminal AMH and spermatogenesis.

In the present study, we measured seminal AMH concentrations in oligozoospermic men and normal men, examining correlation between AMH and various clinical parameters.

Materials and methods

Thirty-nine oligozoospermic men were studied for at least 2 years. Ages ranged from 26 to 44 years (mean ± SD, 32.7 ± 4.3). Patients were evaluated clinically according to World Health Organization protocol (World Health Organization, 2000). Semen analyses were performed according to the World Health Organization Laboratory Manual (World Health Organization, 2000). Semen samples were obtained by masturbation after at least 5 days of abstinence. Testicular volume was measured using an orchidometer. Testicular biopsies were performed bilaterally after informed consent. Pathological findings in the biopsy specimens were scored according to Johnsen (Johnsen, 1970). Ten volunteers with normal seminograms were studied as controls.

Serum hormones and AMH assays

Serum samples were obtained from all patients and stored at −20°C until assays. FSH and LH were determined by chemiluminescence assays (Bayer, Wuppertal, Germany). Testosterone and estradiol were measured by radioimmunoassay (Yatoron, Tokyo, Japan). Detection limits were 0.3 mIU/ml for FSH, 0.1 mIU/ml for LH, 0.05 ng/ml for testosterone and 10 pg/ml for estradiol. Seminal plasma was obtained by centrifugation after liquefaction, frozen quickly and then stored at −80°C until assays. AMH was measured using a commercially available double-antibody enzyme-linked immunoassay (Serotec, Oxford, UK). Intra- and inter-assay coefficients of variation were 6 and 15% respectively. The lowest detectable AMH concentration was 1.8 pg/ml.

Statistical analysis was performed using the Statview statistical program for Macintosh (Abacus Concepts Inc., Calabasas, CA, USA). Spearman’s rank correlation test was used to assess correlation and the non-parametric Mann–Whitney test was used to assess differences.
Correlation between seminal AMH concentration and sperm concentration \( (r = 0.339, P = 0.0350) \).

Table I. Patients characteristics and seminal anti-Müllerian hormone (AMH) level

<table>
<thead>
<tr>
<th></th>
<th>Oligozoospermic men</th>
<th>Normal men</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>32.7 ± 4.3</td>
<td>25.8 ± 2.3</td>
</tr>
<tr>
<td>Sperm concentration ( \times 10^6/ml )</td>
<td>9.2 ± 6.4</td>
<td>110 ± 47.5</td>
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<tr>
<td>Sperm motility (%)</td>
<td>37.9 ± 20.6</td>
<td>59.6 ± 13.1</td>
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<tr>
<td>Sperm morphology (% normal form)</td>
<td>53.2 ± 11.8</td>
<td>51.5 ± 13.9</td>
</tr>
<tr>
<td>Testicular volume (ml)</td>
<td>17.8 ± 4.5</td>
<td>22.9 ± 2.3*</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>7.9 ± 5.3</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>4.1 ± 1.9</td>
<td>2.8 ± 1.4</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>6.5 ± 8.3</td>
<td>4.4 ± 1.8</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>25.7 ± 11.9</td>
<td>36.1 ± 6.1</td>
</tr>
<tr>
<td>Johnsen’s score</td>
<td>6.7 ± 0.9</td>
<td>–</td>
</tr>
<tr>
<td>Seminal AMH (pmol/l)</td>
<td>140.3 ± 254.0</td>
<td>249.0 ± 167.7*</td>
</tr>
</tbody>
</table>

\*P < 0.05.

between groups. \( P < 0.05 \) was regarded as indicating a significant difference.

Results

Seminal analysis data, seminal AMH, testicular volume and serum hormone concentrations are compared between oligozoospermic men and controls in Table I. Seminal AMH concentrations in oligozoospermic infertile patients and controls were (mean ± SD) 140.3 ± 254.0 and 249.0 ± 167.7 pmol/l, representing a significant difference \( (P = 0.0337) \). The seminal concentration of AMH correlated significantly with sperm concentration \( (r = 0.339, P = 0.0350) \) and with total testicular volume \( (r = 0.440, P = 0.246) \), but not with sperm motility. No correlation was seen between seminal concentration of AMH and Johnsen’s score. The serum LH concentration correlated significantly with the seminal AMH concentration \( (r = -0.365, P = 0.0241) \). Serum concentrations of FSH, testosterone and estradiol showed no relationship with seminal AMH concentration.

Discussion

Serum AMH concentrations have been investigated in many reports, but very little has been determined about the significance of seminal AMH concentrations. In this study, we measured AMH in seminal plasma from oligozoospermic and normal men to examine the association between sperm production and seminal AMH.

In humans, large amounts of AMH are produced during fetal and post-natal testicular development (Tran et al., 1987; Voutilainen and Miller, 1987; Kuroda et al., 1990; Sweeney et al., 1997). AMH expression is sharply down-regulated at the time when primary spermatocytes appear. This decrease in AMH expression reflects terminal differentiation of Sertoli cells and is probably only partially dependent upon a regulatory factor associated with the onset of meiosis (Meyts et al., 1999). While some AMH is secreted after puberty, in adults the concentrations of AMH are significantly higher in seminal plasma than in serum (Fenichel et al., 1999). This suggests that AMH is preferentially secreted at the apical pole of the Sertoli cell, toward the seminiferous tubular lumen (Fallat et al., 1996; Fenichel et al., 1999). Maddocks and Sharpe also reported a progressive reduction with age in the secretion of
Inhibin-α via the base of Sertoli cells into the interstitium and an increase via the apex of the Sertoli cells (Maddocks and Sharpe, 1990). The mechanism of the directional change remains unknown. Baarends et al. suggested that the presence of more developmentally advanced spermatogenic cells may direct AMH secretion toward the Sertoli cell apex in relation to specific maturation stages of the seminiferous epithelium (Baarends et al., 1995).

In the present study, we demonstrated that the seminal AMH concentration correlated with sperm concentration and that the seminal concentration of AMH in normal control subjects was significantly higher than in oligozoospermic men. Fenichel et al. also demonstrated that, in non-obstructed azoospermia, the seminal AMH concentration was lower than in fertile donors (Fenichel et al., 1999). Therefore, we believe that the seminal AMH concentration is a marker of spermatogenesis, and that seminal AMH secreted from the apical aspect of Sertoli cells may be involved in sperm production and germ cell proliferation. Although Fallat et al. demonstrated an inverse relationship between seminal AMH concentration and motility index (Fallat et al., 1996), we did not find that relationship.

While a low AMH concentration in seminal plasma appears to be related to spermatogenic dysfunction, a low concentration also may suggest immaturity of Sertoli cells. A Sertoli cell with immature characteristics cannot secrete AMH through its apical layer into seminiferous tubules; instead, any secretion proceeds through the basal layer into the interstitium. As a consequence, seminal concentrations in mature Sertoli cells are higher than in serum, and than those in seminal plasma derived from immature Sertoli cells. The seminal concentration of AMH could serve as a marker of Sertoli cell maturity. In addition, severe spermatogenic impairment in human seminiferous tubules is associated with a population of Sertoli cells that exhibit a prepubertal stage of development (Steger et al., 1996). The seminal concentration of AMH should be helpful for determining the extent to which Sertoli cell immaturity is associated with defective spermatogenesis or contributes to spermatogenic dysfunction.

In conclusion, seminal concentration of AMH correlates with sperm production and may be a good marker for spermatogenesis, as well as Sertoli cell development.

References

Submitted on September 7, 2001; accepted on November 21, 2001