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·*Clinical Experience*·

Seminal plasma anti-Müllerian hormone level correlates with semen parameters but does not predict success of testicular sperm extraction (TESE)

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Abstract

Aim: To assess seminal plasma anti-Müllerian hormone (AMH) level relationships in fertile and infertile males. **Methods:** Eighty-four male cases were studied and divided into four groups: fertile normozoospermia (n = 16), oligoasthenoteratozoospermia (n = 15), obstructive azoospermia (OA) (n = 13) and non-obstructive azoospermia (NOA) (n = 40). Conventional semen analysis was done for all cases. Testicular biopsy was done with histopathology and fresh tissue examination for testicular sperm extraction (TESE) in NOA cases. NOA group was subdivided according to TESE results into unsuccessful TESE (n = 19) and successful TESE (n = 21). Seminal plasma AMH was estimated by enzyme linked immunosorbent assay (ELISA) and serum follicular stimulating hormone (FSH) was estimated in NOA cases only by radioimmunoassay (RIA). **Results:** Mean seminal AMH was significantly higher in fertile group than in oligoasthenoteratozoospermia with significance (41.5 ± 10.9 pmol/L *vs.* 30.5 ± 10.3 pmol/L, $P < 0.05$). Seminal AMH was not detected in any OA patients. Seminal AMH was correlated positively with testicular volume ($r = 0.329$, $P = 0.005$), sperm count *($r = 0.483$, $P = 0.007$)*, sperm motility percent ($r = 0.419$, $P = 0.021$) and negatively with sperm abnormal forms percent ($r = –0.413$, $P = 0.023$). Nonsignificant correlation was evident with age ($r = –0.155$, $P = 0.414$) and plasma FSH ($r = –0.014$, $P = 0.943$). In NOA cases, seminal AMH was detectable in 23/40 cases, 14 of them were successful TESE (57.5%) and was undetectable in 17/40 cases, 10 of them were unsuccessful TESE (58.2%). **Conclusion:** Seminal plasma AMH is an absolute testicular marker being absent in all OA cases. However, seminal AMH has a poor predictability for successful testicular sperm retrieval in NOA cases. *(Asian J Androl 2007 Mar; 9: 265–270)*

Keywords: seminal plasma; anti-Müllerian hormone; spermatogenesis; azoospermia; testicular sperm extraction

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1 Introduction

Anti-Müllerian hormone (AMH) or Müllerian inhibiting substance (MIS) is produced by the Sertoli cells of the prenatal testis and lasts throughout life [1]. In the

male embryo, it is responsible for regression of the Müllerian duct. Its production does not cease after the fetal period and it is present in the serum of adult men [2]. Fénichel *et al*. [3] showed that high concentrations of AMH were detectable in the seminal fluid of fertile men but absent in all obstructive azoospermic specimens. Fujisawa *et al*. [4] found that seminal AMH is a good marker for Sertoli cell development and that it correlated significantly with sperm concentration, testicular volume, serum luteinizing hormone (LH) but not with serum follicular stimulating hormone (FSH), testosterone or estradiol. Al-Qahtani *et al*. [5] found that seminal AMH concentrations in male factor infertility were not significantly different from fertile men, where serum AMH was lower in male factor infertility than that in fertile cases.

In non-obstructive azoospermia (NOA), minute foci of spermatogenesis if present could be used successfully for intracytoplasmic sperm injection (ICSI). However, testicular sperm extraction (TESE) may not always be successful in all NOA cases. Therefore, determination of factors that can predict a successful sperm recovery procedure can offer realistic expectations for both the couple and the physician [6]. Different criteria were suggested as predictive markers of TESE outcome; clinical, laboratory or histopathological [7–8]. Several markers have already been proposed, e.g. transferrin [9], lactate dehydrogenase [10], insulin-like growth factor (IGF)-1 [11] and inhibin B [12]. However, no one could foretell about the presence of a spermatogenic focus inside an NOA testis regarding TESE/ICSI processes, especially in border-line conditions such as small-sized testicles and aged female partner.

In NOA, Fénichel *et al*. [4] found that undetectable seminal AMH was associated mostly with lack of testicular spermatozoa retrieval, while detectable seminal AMH was associated with persistent spermatogenesis in 70% of cases with a negative predictive value of 83%.They suggested that seminal AMH may represent a non-invasive marker of spermatogenesis in NOA, which may indicate the likley success of testicular sperm recovery before ICSI.

In the present work, we assessed the diagnostic value for seminal plasma AMH estimation in different groups of male infertility.

2 Materials and methods

2.1 Patients

Eighty four male cases (mean age 35.5 ± 7.6 years, range 25–37 years) were recruited consecutively, after consent and Institutional Review Board (IRB) approval, from the Andrology & Sexology Department, Faculty of Medicine Cairo University Hospital (Cairo, Egypt) and Adam International Clinic (Giza, Egypt). They were divided into four groups: fertile normozoospermia (n=16), oligoasthenoteratozoo-spermia (n = 15), obstructive azoospermia (OA) (n = 13) and NOA with normal 46 XY karyotype (n = 40). They were subjected to history taking, general and genital examinations and semen analysis. Serum FSH assays and testicular biopsy were performed for NOA cases. OA cases were selected from those scheduled for epididymo-vasostomy operations or post-vasectomy cases. The NOA group was further subdivided according to TESE trials into unsuccessful and successful TESE subgroups.

2.2 Samples

Semen samples were collected after a period of sexual abstinence for 4 to 5 days. All samples were retrieved in a specimen container and allowed to liquefy for 30 minutes over a slide warmer at 37ºC. A routine semen analysis was then performed according to World Health Organization (WHO) guidelines [13], azoospermia was verified after two semen analyses and centrifugation. Semen samples were centrifuged at 100 × g for 15 min and the supernatant seminal plasma was stored in polypropylene tubes at –80ºC until used.

2.3 Testicular biopsy and histopathology

Under anesthesia, a small incision (0.5 cm) was done in the least apparent vascular area to expose testicular tissue. Gentle pressure was applied to extrude a piece of testicular tissue (0.5 × 0.5 cm) microsurgically, excised with sharp scissors and added directly to Bouin's fixative solution. Histological sections were prepared from the paraffin blocks and stained by hematoxylin and eosin stain, then examined under the light microscope. Testicular sections were classified according to the prevailing pattern of spermatogenic cells into: normal spermatogenesis pattern (> 50% of tubules had full spermatogenesis up to spermatozoa), hypospermatogenesis (little number of full spermatogenesis tubules), spermatogenic arrest (none of the tubules with sperms but with spermatids or primary spermatocytes), Sertoli cell only (SCO) (tubules had no germ cells and lined with Sertoli cells only) and tubular atrophy (no seminiferous epithelial cells with tubular sclerosis).

2.4 Fresh testis examination

During testis biopsy procedure, a piece of tissue was put in a Petri dish (Falcon cat no. 300, Becton Dickson, Lakes, NJ, USA) containing 1 mL N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (HEPES) buffered Earl's salt solution and transferred immediately into the adjacent laboratory. The tissues were minced under laminar flow and then examined under an inverted microscope × 400 for the presence of testicular spermatozoa. If no spermatozoa were seen after checking the whole dish, the contents were transferred into a 5-mL falcon tube after removing the testicular debris and centrifuged at 1 800 × *g* for 5 min, then the supernatant was removed and the pellet was rechecked for spermatozoa [14]. The result was interpreted when sperms were seen or not as successful or unsuccessful TESE trials.

2.5 AMH and FSH estimation

AMH was measured (pmol/L) in seminal plasma after centrifugation by ELISA method (DSL, Webster, TX, USA). In the assay, standards, controls, and serum samples were incubated in microtitration wells coated with anti-AMH antibody. After incubation and washing, the wells were treated with secondary anti-AMH detection antibody labeled with biotin. After a second incubation and washing step, the wells were incubated with streptavidin-horseradish peroxidase (HRP). After a third incubation and washing step, the wells were incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm. The minimum detection limit was 3.0 pmol/L with an intra-assay coefficient of variation (CV) 2.4–4.6%, and an inter-assay CV 4.8–8.0%. Serum FSH (mIU/mL) was estimated using radioimmunoassay (RIA) with sensitivity of 0.06 mIU/mL, an intra- and inter-assay CV < 10%.

2.6 Statistical analysis

Numerical data were expressed as mean ± SD and range. Comparisons were performed by paired *t*-test. Correlations were tested by Spearman's test. Comparisons and correlations were considered statistically significant if $P < 0.05$.

3 Results

Normozoospermic fertile men showed mean testicular volume of 16.8 ± 1.6 mL, mean sperm concentration 66.2 ± 45.1 × 10^6/mL, mean sperm motility 56.9 ± 6.0%, mean sperm abnormal forms 26.3 ± 5.0%. oligoasthe-noteratozoospermic men had mean testicular volume of 13.1 ± 1.6 mL, mean sperm concentration 3.9 ± 2.2 × 10^6/mL, mean sperm motility 20.0 ± 8.5%, mean sperm abnormal forms 74.7 ± 9.9%. Mean seminal AMH in the fertile group was significantly higher than that in oligoasthenoteratozoospermia (41.5 ± 10.9 pmol/L *vs.* 30.5 ± 10.3 pmol/L, $P < 0.05$). Seminal AMH was not detected in any OA case and was detected in 23/40 (57.5%) of NOA cases (Table 1). Seminal AMH was correlated positively with testicular volume ($r = 0.32$, $P = 0.005$), sperm count ($r = 0.483$, $P = 0.007$), sperm motility percent ($r = 0.419$, $P = 0.021$) and negatively with sperm abnormal forms percent ($r = –0.413$, $P = 0.023$). Nonsignificant correlation was evident with age ($r = –0.155$, $P = 0.414$) and plasma FSH ($r = –0.014$, $P = 0.943$).

NOA unsuccessful TESE cases ($n = 19$) had a mean testicular volume of 9.9 ± 4.6 mL, mean serum FSH of 17.3 ± 9.2 mIU/mL and mean seminal AMH of 14.2 ± 13.5 pmol/L. AMH was detectable in 9/19 cases (47.4%) (Table 2). NOA successful TESE cases ($n = 21$) had a mean testicular volume of 12.6 ± 4.6 mL, mean serum FSH 13.1 ± 10.9 mIU/mL and mean seminal of 23.2 ± 20.6 pmol/L. AMH was detectable in 14/21 cases (66.7%) (Table 3).

Table 1. Comparison between seminal anti-Müllerian hormone (pmol/L) in different groups. [b]$P < 0.05$, compared with fertile group; [c]$P < 0.01$, compared with fertile group.

	Fertile	Oligoasthenozoospermia	Obstructive azoospermia
n	16	15	13
Mean ± SD	41.5 ± 10.9	30.5 ± 10.3[b]	0[c]
Mean ± SE	41.5 ± 2.7	30.5 ± 2.7	0[c]

Table 2. Data for NOA unsuccessful TESE cases. SCO, Sertoli cell only.

No.	Age (years)	Testis volume (mL)	Histopathology	Serum FSH (mIU/mL)	Seminal AMH (pmol/L)
1	44	3	SCO	27.0	3
2	37	5	Tubular atrophy	30.0	3
3	35	3	Tubular atrophy	22.8	3
4	38	11	SCO	22.7	25.0
5	40	6	SCO	7.7	17.5
6	35	12	mixed SCO	33.0	10
7	32	5	SCO	7.1	27.0
8	30	14	SCO	9.5	30.0
9	40	6	SCO	8.4	3
10	33	12	SCO	11.9	3
11	30	15	spermatogenic arrest	9.5	3
12	41	6	tubular atrophy	20.3	3
13	39	12	SCO	21.0	28
14	35	13	tubular atrophy	12.2	3
15	36	18	mixed SCO	7.2	3
16	37	6	tubular atrophy	30.8	3
17	43	12	SCO	15.9	32.5
18	32	13	mixed SCO	26.3	42.0
19	32	16	mixed SCO	5.9	27.5
Mean ± SD	36.3 ± 4.2	9.9 ± 4.6		17.3 ± 9.2	14.2 ± 13.5

Table 3. Data for NOA successful TESE cases. SCO, Sertoli cell only.

No.	Age (years)	Testis vol. (mL)	Histopathology	Serum FSH (mIU/mL)	Seminal AMH (pmol/L)
1	40	7	hypospermatogenesis	2.5	3.0
2	32	16	tubular atrophy	2.0	18.5
3	29	5	SCO	25.5	3.0
4	35	2	SCO	36.0	68.0
5	25	11	spermatogenic arrest	5.1	3.0
6	32	7	hypospermatogenesis	8.9	66.0
7	30	14	hypospermatogenesis	8.3	27.5
8	42	18	spermatogenic arrest	2.6	28.5
9	35	16	hypospermatogenesis	12.8	40.0
10	50	14	spermatogenic arrest	5.5	47.5
11	38	15	Mixed SCO	10.4	27.5
12	52	16	spermatogenic arrest	7.2	32.5
13	41	18	tubular atrophy	20.7	38.5
14	52	13	hypospermatogenesis	9.0	3.0
15	49	13	spermatogetic arrest	10.0	3.0
16	33	16	hypospermatogenesis	6.6	32.5
17	35	17	hypospermatogenesis	27.8	3.0
18	41	6	SCO	40.0	10.0
19	42	15	spermatogetic arrest	18.8	8.5
20	52	10	hypospermatogenesis	7.7	3.0
21	49	15	hypospermatogenesis	7.5	21.8
Mean ± SD	39.7 ± 8.4	12.6 ± 4.6		13.1 ± 10.9	23.2 ± 20.6

5 Discussion

The testes express a high level of AMH, produced by Sertoli cells, from early fetal life driven by the transcription factors SOX9, SF1, WT1 and GATA4, until puberty, when it is downregulated by testosterone and meiosis. When the androgen negative effect is absent, FSH increases the secretion of AMH. Serum AMH estimation was found to be useful in evaluating children with nonpalpable gonads, with or without ambiguous genitalia. It signals the existence of functional testicular tissue and allows a distinction between gonadal dysgenesis and dissociated tubular-interstitial dysfunction. Also, serum AMH was found to be a useful marker in the follow-up of males with precocious puberty or hypogonadotrophic hypogonadism and patients with sex cord stromal tumours of the gonads [15]. Isikoglu *et al*. [16] speculated that studying serum AMH might be more advantageous than seminal plasma because of the presence of seminal proteases. However, there was no difference in serum of AMH levels between the studied groups.

In different studies, seminal AMH was related to sperm motility and testicular spermatogenesis [3, 4, 17]. In the present study, fertile normozoospermic cases had significantly higher AMH levels compared to infertile oligoasthenozoospermia. In addition, seminal AMH was positively correlated with testicular volume, sperm concentration, sperm motility percent and negatively with sperm abnormal forms percent. A nonsignificant correlation was demonstrated with either the age or serum FSH. Fujisawa *et al*. [4] correlated seminal AMH significantly with sperm concentration, testicular volume, serum LH but not with serum FSH, testosterone or estradiol. Fallet *et al*. [17] suggested that AMH may have a function in modulating motility.

In the obstructive azoospermia group (n = 13), AMH was not detectable in all cases confirming its testicular origin. This corresponds with the study of Fénichel *et al*. [3], who observed undetected seminal AMH in their nine obstructive azoospermic studied cases. Total alpha glucosidase was used for a long period to discriminate epididymal obstruction, but it was not valid because of its multi-producing sources [18]. Therefore, seminal AMH may stand as one of the testicular markers. In addition, AMH and alpha-glucosidase could be complementary in their diagnostic values. Normal neutral glucosidase value with no AMH would indicate no obstruction of the epididymal tubule, which has a normal secretory function (seminal vesicle secretes acidic glucosidase) showing possibly a testicular defect or blockage of the efferent ducts.

In NOA cases, seminal AMH was detectable in 23/40 cases, 14 of them were successful TESE, while it was undetectable in 17 cases, 10 of them were unsuccessful TESE. Fénichel *et al*. [3] detected seminal AMH in 9/23 NOA cases, 7 of them gave successful TESE, while it was undetectable in 14 cases, 11 of them gave unsuccessful TESE. This can give a clue as to why seminal plasma AMH alone cannot be used to predict TESE results. Seminal AMH was detectable in cases with advanced spermatogenesis, which corresponds with Baarends *et al*. [19] who demonstrated that the presence of developmentally more advanced spermatogenic cells may enhance AMH secretion, related to the specific stages of the seminiferous epithelium cycles.

NOA cases with unsuccessful TESE (n = 19) showed no detection of seminal AMH in 10/19 (52.6%). Most of these cases were of progressive testicular pathology, small testicles size or with elevated FSH. Undetectable AMH in these cases may follow failed spermatogenic activity or hindered production by different antagonizing testicular paracrine function. A definite threshold of AMH secretion from intact Sertoli cells seems to be required in order to appear in seminal plasma. Fénichel *et al*. [3] showed that the dramatic decrease in seminal AMH was associated with spermatogenic failure, suggesting a link between AMH and spermatogenic hormones. In addition, the possibility that decreased seminal AMH reflects a primary alteration in Sertoli cell function that leads to spermatogenic arrest must not be ruled out. Nine cases of this group (47.4%) showed seminal AMH that may be because in unsuccessful TESE a lack of spermatozoa in one or more sites does not guarantee a complete lack of sperms in the whole testis [4, 20]. This sheds light on the fact that seminal plasma AMH alone cannot foretell the absence of spermatogenic focus inside the testis.

In conclusion, seminal plasma AMH may stand as one of the testicular markers. However, it has a poor predictability for successful testicular sperm retrieval in NOA cases.

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