

# Role of anti-Mullerian Hormone and Gamma-glutamyl-transpeptidase in the Sera and Seminal Plasma in Infertile Men in Baghdad

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## Abstract

The study was carried out through the period from February /2015 to June 2015, for estimation the concentration of anti-Mullerian hormone ( AMH) in the seminal plasma & sera of infertile males compared with healthy group as control , also the gamma-glutamyltranspeptidase level were measured in the seminal plasma and sera of patients and healthy , (79 ) infertile male and ( 32 ) healthy , with age ranged from( 18 – 49 ) year for both groups .

The diagnosis done by macroscopic and microscopic examination of semen according to W.H.O. standard criteria. A significant decrement ( $P < 0.05$ ) was observed for male with oligozoospermia as compared to other groups of male infertility factor. There was significance elevation ( $P < 0.05$ ) for male complaining from teratozoospermic as compared to normozoospermic , oligozoospermic and a zoospermia.

Similarly, significant Increment ( $P < 0.05$ ) in seminal plasma (AMH) for male suffering from oligozoospermia compared to other groups. Meanwhile teratozoospermic patients showed significant increment  $P < 0.05$  as compared with other infertile groups. Also azoospermic patients revealed significant reduction  $P < 0.05$  in the concentration of seminal plasma AMH compared with other infertile group.

Normozoospermic male showed a significant ( $P < 0.05$ ) elevation in the concentration of GGT when compared with other infertile groups . Teratozoospermic patients showed a significant decrement ( $P < 0.05$ ) in the concentration of GGT . A theuozoospermic patients showed significant elevation ( $p \leq 0.05$ ) of concentration of seminal fluid GGT, while oligozoospermia patients significant decrement ( $P < 0.05$ ) as compared with other infertile groups .

**Keywords:** Anti-Mullerian hormone, gamma-glutamyl- , transpeptidase , infertile males .

## الخلاصة

شملت هذه الدراسة (79) مريضاً مصاباً بالعقم الأولي والثانوي ، بالإضافة الى (32) شخصاً سليماً تم اعتبارهم مجموعة سيطرة، تراوحت اعمار المجموعتين بين (18- 49) سنة للفترة من شباط 2015 الى حزيران 2015 في منطقة الرصافة لمحافظة بغداد. ركزت هذه الدراسة على تشخيص السائل المنوي للرجال المرضى والاصحاء عيانياً ومجهرياً اعتماداً على معايير منظمة الصحة العالمية لعام 2010. تم قياس الهرمون المضاد لقناة مولر في السائل المنوي للمرضى والاصحاء وكذلك في مصولهم ، بالإضافة الى قياس تركيز الكاماكلوتاميل ترانس بيتايديز في السائل المنوي ومصول المرضى والاصحاء . لوحظ وجود انخفاض معنوي ( $p < 0.05$ ) في مستوى الهرمون المضاد لقناة مولر في مجموعة قلبي النطف عند مقارنتها بمجاميع العقم الاخرى . كما شوهد ارتفاعاً في هذا الهرمون ( $p < 0.05$ ) لمجموعة العقم التي تعاني من قلة الحركة والمجموعة التي تعاني من تشوهات في الشكل بالمقارنة مع مجاميع الذكور طبيعية النطف . اما في ما يتعلق بمستوى الكاما-كلوتاميل ترانس بيتايديز ، فقد اظهرت مجموعة الذكور طبيعية النطف زيادة معنوية ( $p < 0.05$ ) في مستوى انزيم (GGT) في مصول المرضى مقارنة مع مجاميع العقم الاخرى . في حين هناك انخفاض معنوي ( $p < 0.05$ ) لمستوى الانزيم في مصول المرضى مشوهي اشكال النطف بالمقارنة مع مستواه في مجاميع العقم الاخرى. لوحظ انخفاضه معنويًا ( $p < 0.05$ ) في مستوى هذا الانزيم لمجموعة قلبي العدد بالمقارنة مع تركيزه في مجاميع العقم الاخرى .

**الكلمات المفتاحية:** الهرمون المضاد لقناة مولر , انزيم كاما كلوتاميل – ناقل البيبتيد , الرجال العقيمين

## Introduction

Anti-Mullerian hormone (AMH) is a 140 Kilodalton glycoprotein that is produced during normal embryogenesis by the sertoli cells (SC) of the embryonic testis . It causes the evolution of the Mullerian duct and inhibits female gonadogenesis by producing apoptosis of target gonadal cells , the main reproductive organs of the male are the testes , which generate spermatozoa and two different hormones of normal male sexual discrimination Anti-Mullerian hormone and testosterone (Rey, 2000; Fujisawa *et al.*,2002).

The testes expressed AMH at the 8<sup>th</sup> week of gestation and still produced at high grade up to puberty , decrement of AMH production is characterized when sertoli cells maturation(Matuszczak *et al.* ,2013).Probably the increment of AMH increase is related to follicular stimulating hormone (FSH) –induced (SC) proliferation, and also activation of AMH gene transcription although passage way mediated by cyclic a adenosine mono-phosphate (CAMP) (Hermanowicz *et al.*, 2013).

AMH increase in concentration in boys in the first month reaching a highest level at 6<sup>th</sup> month of age and then decrease gradually through childhood reaching to minimal level in tens (Lee *et al.*,2003) . However at puberty AMH declines as a result from gradual activation of hypothalamic –pituitary gonadal axis, and follow increase intra-testicular testosterone (Hero *et al.*, 2012) .

The AMH gene is situated on chromosome 19, the synthesization of human AMH as 560 amino acid precursor with 24-25 amino acid leader involving 16 – 18 amino acid signal chair and delusive 7-8 remains pro-sequence (Cate *et al.*, 1986).In the first day after birth SC specific peptides inhibin-B and AMH are at their lowest levels but raise once again the first week, probably reflecting dynamic SC abundance (Bergada *et al.*, 2006).

This AMH increase is probably associated to FSH induced SC production, and also activation of AMH gene transcription through a pathway mediated by CAMP (Rey *et al.*, 2003) The AMH declined with increasing age, but in contrast, the FSH, LH and testosterone increased with increasing age (Aksklaede *et al.*,2010)

AMH appears to bind directly to sperm, but AMH receptor type -II is not expressed in sperm (Fallat, 1996). The defects in AMH may be a results of various conditions with low,normal or high AMH level may be belong to different factors, persistent Mullerian duct syndrome, congenital hypogonadotropic hypogonadism, cryptorchidism and varicocele.(Matuszczak *et al.*,2013)

Gamma-glutamyl-transpeptidase( GGT ) is an enzyme produced in the bile ducts, is a cell-surface protein contributing to the extra-cellular catabolism of glutathione, also produced in many tissues, but most GGT in serum is derived from liver (Emdin *et al.*,2005) The obesity, alcohol drinking and cigarette smoking are completely connected with raised serum GGT and that coffee consumption is inversely related to raised serum GGT (Nakanishi *et al.*,2010).

Seminal GGT is produced mostly from prostate gland and is around 200 times upper than that of blood (Uchijima *et al.*, 1986). GGT genes of human are located on chromosome 22 about seven or more genes of GGT in humans (Chicki *et al.*, 1999). GGT actively of normal human seminal plasma and prostatic fluid is 500-800 folds higher than the activity noted in normal human serum (Abe *et al.*, 1991).

### **Aim of the Study**

To estimate the AMH level in the seminal fluid and sera of infertile male compared with healthy control, as well as measurement of GGT concentration in infertile patients in all groups.

### **Materials and Methods**

#### **Semen analysis:**

Semen was done according to the W.H.O. criteria (w.H.O, 2010). Ninety seven infertile men with aged 18-49 years and 32 healthy control at the period from February 2015 –June /2015 at Al-Rusafa sector/Baghdad governorate .The ejaculates were collected after abstinence period of 3 days in a sterile non-toxic disposable container and put in incubator at 37C° , .

The semen samples are examined ,macroscopically (semen appearance, semen volume, semen liquefaction and semen PH), then also microscopically examined (semen concentration , sperm motility , sperm agglutination , sperm morphology and round cells) .

#### **Seminal Plasma Preparation**

The semen samples were centrifuged for 15 minute at 2600 rpm , the supernatant (seminal plasma ) was stored at (-20 C°) to be measured level of AMH was specified by using ELISA technique , while GGT were determined using enzymatic method . The infertile males control were divided into 5 groups according to their sperm concentrations (normozoospermic , a zoospermic , oligozoospermic , asthenozoospermic and teratozoospermic) .

#### **Blood Sample Collection**

Three milliliter of venous blood was aspirated from each male, collected in a plain tube, allows clotting, and then centrifuged at 3000 rpm for 5 minutes. The sera samples were stored at (-20) C° to measure AMH by ELISA Technique, while GGT was determined by enzymatic method. The data was statistically analyzed using SPSS/PC version 18 software (SPSS), Chicago.

#### **Assay Procedure of AMH**

All specimens and reagents were allowed to be at room temperature and mix gently before use.

1. In each well about 25uL of the calibrators, control was placed.
2. AMH assay buffer, 100uL then was added to each well.
3. The plates were incubated, shaken gently for 90 min. at 37C° incubator.
4. All plates were aspirate and wash five times with washing solution.
5. Antibody –biotin conjugates RTU, 100uL then was added to each well.
6. The plates were incubated with shaking gently for 30 min. at 37C°.
7. Then was aspirate and wash five times with washing solution.
8. Streptavidin – enzyme conjugate –RTU, 100uL was added to each well.
9. The plate was incubated and shaking gently for 30 min. at 37C°.
10. Again it was aspirate and wash five times with washing solution.
11. TMB chromosome solution, 100uL was added to each well.
12. The wells were incubated, shaking gently for 12 min. at 37C°.
13. Stop solution, 100uL then was added to each well.

The absorbance of the solution in the wells was read at 450 nm within 30 minutes.

**Results**

Table (1) shows semen parameters for primary and secondary infertility according to W.H.O. criteria ( 2010 ). There are non-significant differences were observed between primary and secondary infertility for the semen parameters ( semen volume ,liquefaction time , semen PH , sperm motility % , while sperm concentration was observed increment significant)  $P < 0.05$  between primary and secondary infertility .

**Table (1): Sperm parameters for infertile patients with primary and secondary infertility according to W.H.O. criteria.**

Sperm parameters	Types of fertility		W.H.O criteria	P. value
	Primary 61 (77.3%)	Secondary 18(22.7%)		
Volume of sperms / (mL)	2.29± 0.12	2.17±0.19	1.5-5mL	0.89
Ph of semen	7.81±0.04	7.77±0.03	7.2-7.8	0.87
Agglutination of sperm%	1.21±0.55	1.16±0.61	< 10%	0.93
Morphology of normal sperms%	26.93±1.46	27.61±1.88	≥ 30%	0.39
Liquefaction time of semen / min.	53.31±1.69	47.77±1.93	Within60 Min.	0.34
Motility of sperm%	22.23±1.93	19.17±2016	≥ 32%	0.49
Conc. of sperm (million/ ml)	26.31±2.73	30.26±2.51	≥15million/ml	0.05*

\*Significant value

While semen parameters of healthy (normozoospermicmale ) group ( 32 male ) were shown in table (2) .

**Table (2): Sperm parameters of healthy control.**

Volume of sperms / ( ml)	2.93±0.19
PH of semen	7.53±0.03
Agglutination of sperm%	2.93±1.01
Morphology of normal sperms%	39.22±0.61
Liquefaction time of semen / min.	24.34±2.77
Motility of sperm%	42.31±3.11
Conc. Of Sperm/million mL	49.98±3.91

Table (3) show the level of AMH and GGT in sera and seminal plasma of (normozoospermic male) ,the level of sera MAH showed high significant differences ( $p < 0.01$ ) compared with level of seminal plasma of same parameters , also there is high significant of the level of sera GGt compared with seminal plasma.

**Table (3): Concentration of seminal plasma and sera AMH and GGT in normozoospermic male.**

<i>Parameter</i>	<i>Seminal plasma</i>	<i>Sera</i>	<i>p. Value</i>
<i>AMH ng/Ml</i>	<i>3.33±1.22</i>	<i>6.91± 0.77</i>	<i>0.00**</i>
<i>GGT iu/L</i>	<i>3124±14.19</i>	<i>21.13±7.8</i>	<i>0.001**</i>

\*\* high Significant value

Table (4) shows the concentration of AMH and GGT in seminal plasma and sera of azoospermic patients, there were highly significant differences ( $p < 0.01$ ) in the concentration of AMH and GGT

**Table (4): Concentration of seminal plasma and sera of AMH and GGT in azoospermic patients.**

Parameter	Seminal plasma	Sera	P. value
AMH ng/mL	2.01±0.04	6.81±0.63	0.001**
GGT Iu/L	3169.33±305	13.49±1.21	0.00**

\*\* high Significant value

Table (5) shows the concentration of AMH and GG in seminal plasma and sera of oligozoospermic patients, there was significant difference ( $p < 0.05$ ) in the concentration of AMH in seminal plasma and sera, while highly significant difference compared with the concentration of seminal plasma and sera of same parameter.

**Table (5): Concentration of seminal plasma and sera of AMH and GGT in oligozoospermic patients.**

Parameter	Seminal plasma	Sera	p. value
AHM ng/ mL	6.73±1,99	3.11±0.61	0.036**
GGT Iu/L	2987.51±409.12	14.43±2.22	0.001**

\*\*high significant value

Table (6) shows the concentration of AMH and GGT in asthenozoospermic patients, there are significant difference compared with level of seminal plasma, while GGT concentration was highly significant, while GGT concentration was highly significant differences ( $p < 0.01$ ) in same parameters.

**Table (6): Concentration of seminal plasma and sera of AMH and GGT in asthenozoospermia patients.**

Parameter	Seminal plasma	Sera	P. value
AMH ng/mL	4.92±1.13	10.31±2.79	0.014**
GGT Iu/L	3943±264.31	12.22±1.91	0.001**

\*\*high significant value

Table (7) shows the concentration of AMH and GGT in terazoospermic patients, there was significance ( $p < 0.05$ ) in the concentration of AMH sera compared to the seminal plasma, while GGT shows highly significant difference ( $p < 0.01$ ) in the sera compared with seminal plasma and sera of AMH and GGT in teratozoospermic patients.

**Table (7): Concentration of seminal plasma and sera of AMH and GGT in teratozoospermic patients.**

Parameter	Seminal plasma	Sera	P. value
AMH ng/mL	5.39±1.73	9.23±2.61	0.027*
GGT Iu/L	3907±466.22	9.71±0.31	0.001*

\* significant value

## Discussion

Since the semen consists of a heterogeneous mixture of sperm with variable motility, sometimes agglutinated, together with leucocytes, germinal cells, amorphous materials and germinal cells, it is necessary to prepare a sperm sample that contains mainly sperm of normal confirmation and progressive mobility (Pareek *et al.* ,2007)

This study shows the percentage of primary infertile males 77.3%, while the secondary infertile males 18.78%, this agreed with the result of (Al-Joubori, 2013; Al-Najjar,2014), that had been in Iraq, also in Egypt 70.7%) of couples had primary infertility and 29.3% had secondary infertility (Serour ,2008)

Many males in reproductive age are unable to get married due to weakness of financial situation that lead to high average age of marriage which is fastened that important cause of infertility, in addition to many males hurled in wars exposed to different types of chemical, weapons which caused negatively on fertile status(Haleem *et al.* ,2014)

There was significant increment in the level of sera AMH ( $p < 0.05$ ) in all fertile duration groups (azoospermia), oligozoospermia, asthenozoospermia and teratozoospermia)

It is may be due to that AMH correlate negatively with sera testosterone concentration, this correlation persists if androgen levels are abnormally high , but gonadotropin are low(Young *et al.* ,1999)

The level of sera AMH it was at its lowest level in Oligoasthenoteratozoospermic and oligozoospermic patients, also the level of seminal plasma AMH was at its lowest in Oligoasthenoteratozoospermic, asthenoteratozoospermic and azoospermic groups with that the significant increment of seminal AMH in oligozoospermic patients compared with the sera of same group (Salih *et al.*, 2014).

After puberty, AMH is released preferentially by the apical pole of Sertoli cell towards the lumen of the somniferous tubules, resulting in higher concentrations in the seminal plasma than in the sera. (Al-Qahtani *et al.* ,2005)

It cell known that GGT in seminal fluid is secreted mainly from the prostate gland and is approximately 200 times higher than that of blood(Uchijima *et al.*, 1986)

Serum GGT was related to alcohol consumption, cigarette smoking, age and body mass index were positively associated with serum GGT level, among clinical variables, systolic and diastolic blood pressure, fasting insulin and total cholesterol showed positive correlation with baseline GGT concentration (Lee *et al.*, 2004)

There was significant decrement ( $p < 0.05$ ) in the concentration of seminal plasma GGT in oligozoospermic patients when compared with the concentration of seminal plasma GGT of other infertile groups. The low concentration of GGT observed in oligozoospermic might be attributed to disturbances in the excretory function of the prostate gland which is agreed with (Awadalla *et al.* ,2003)

Low level of GGT in seminal plasma is a good marker for the detection of infection in the accessory genital glands(Comhaire *et al.*,1989).

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