The association between male serum anti-mullerian hormone and the outcomes of intracytoplasmic sperm injection

Sahib Yahya Hasan Al-Murshidi1, Rabab Zahir Al-Yasiry2, Ali Ibrahim Rahim3 and Said Aldeen Alisawi4

1Department of Urology and infertility, Medical College, Kufa University, Iraq, 2Department of anatomy and histology, College of Medicine, University of Babylon, Iraq, 3Department of anatomy, College of Medicine, University of Kufa 4Department of Urology and infertility, Medical College, Kufa University, Iraq

Email: sahib.almurshidi@uokufa.edu.iq

Abstract. To evaluate the correlation between male serum anti-mullerian hormone and ICSI outcomes (fertilization rate, cleavage rate, quality of embryos, and pregnancy rate). The type of study was controlled randomized study on fifty five infertile couples undergoing ICSI procedure at fertility center in Al-Sadder medical city in Al Najaf Al Ashraf. The research included two groups are unexplained (n=24) and male factor infertility (n=31) who underwent intracytoplasmic sperm injection programme. Blood samples were taken from husbands to assess the amount of anti-mullerian hormone at day of retrieval of follicles. The results of ICSI were reported in both two groups. The correlation between male serum anti-mullerian hormone with fertilization rate, cleavage rate, and quality of embryos was studied. The current research results were revealed significant increase (P <0.001), in concentration of male serum anti-mullerian hormone in unexplained group (7.29 ± 0.73) compared to male factor (2.36 ± 0.19) group. Good quality embryos had significant increase (P value 0.053) in unexplained group (5.70 ± 0.94) compared to male factor group (3.70 ± 0.5). Pregnancy rate was also found to be not significantly differed but the higher percentage of pregnancy (32.3%) occurred in male factor group compared to unexplained group (25%). There was positive correlation (r= 0.293 p=0.03) between good quality embryos and male serum anti-mullerian hormone. Results of this study showed found positive correlation between male serum anti-mullerian hormone and good embryos which may lead to improvement in pregnancy rates.

Key word: anti-mullerian hormone, ICSI outcomes, and good embryos.

Introduction

Anti-mullerian hormone (AMH), also known as mullerian inhibiting substances (MIS), is homodimeric glycoprotein connected by disulfide bonds and has molecular weight 140 kDa, belongs to transforming growth factor beta super family (Cate et al., 1986). In male AMH increases rapidly after birth, its highest during infancy and then gradually decrease until puberty (Lee et al., 1996). The alteration of AMH production during male fetus development might be
classified into four stages: fetal and early postnatal period, childhood, puberty and adulthood (Grinpson and Rey, 2010). In this period, hypothalamus produces GnRH which stimulates release of gonadotropins (LH and FSH). LH acts on Leydig cell receptor of fetus to stimulate testosterone production. Testosterone induces Wollfian duct development and inhibition of AMH production from Sertoli cell by binding to androgen receptor but because of insufficient expression of androgen receptor the latter effect of testosterone dose not appear (Grinpson and Rey, 2010). On the other hand, FSH stimulates AMH expression by binding on its receptor in Sertoli cell membrane (Rey et al., 2009). In this stage, serum levels of AMH are high comparable with childhood AMH level. It is a rest period of HPG axis and is also called the period of “hypo-gonadotropic hypogonadism”. Small amounts of testosterone are produced by Leydig cells and about half of which produced by adrenal gland. Sertoli cells are still immature and spermatogenesis is arrested in pre-meiotic stages. Sertoli comprises a major portion of testicular tissue and FSH stimulation produces small amount of AMH compared with prenatal period (Rey et al.,2008; Grinpson and Rey, 2010). In this stage, is introduced into cytoplasm of mature oocyte. It was discovered in 1992 and used to improve the fertilization in infertile couple with male factor reason or when IVF fails to achieve fertilization (Palermo et al.,1999). It is used for treatment of many causes of male infertility because of its safety and efficacy (Tournaye et al., 2002). Today, ICSI has become the most commonly applicable micromanipulator technique of all IVF procedure. About 60% of IVF processes world wide were performed by ICSI since 2004 (Anderson et al., 2008). The treatment of male factor infertility by using ICSI has been increased from 84% in 2003 to 87% in 2008 (Shridharani and Sandlow, 2010). Use of ICSI in men with normal or borderline semen parameters is widely performed due to many possibilities including: poor oocyte quality, low responder female, unexplained infertility, advanced age of female partner, failure of previous trying of conventional IVF and as routine practice in IVF laboratory (Jain and Gupta, 2007). ICSI has been used for treatment of azoospermic men (Naru et al.,2008) It is used in severe male factor infertility rather than azoospermia when sperm count, motility and morphology are low in such condition the fertilization may not occur when performing conventional IVF (Tehraninejad et al., 2012) Ejaculatory dysfunction, retrograde ejaculation or paraplegic me Infertility of immunological cause: characterized by presence of antibodies in follicular fluid or antisperm antibody in seminal fluid Before treatment of cancer with chemotherapy or radiotherapy (Elder and Dale 2011). Fertilization is confirmed by presence of two pronuclei (2PN) and has been correlated with normal embryo development and first stages of RNA synthesis. It has been documented that fast dividing embryos result in high degree of developmental capacity than slower dividing ones (Shoukir et al., 1997). Transfer of embryos with good implantation competence means that high chance of developing into live baby has been an object of debate and continuous study (Kotze et al., 2014). the current study aimed to evaluate the correlation between male serum anti-mullerian hormone and ICSI outcomes (fertilization rate, cleavage rate, quality of embryos and pregnancy rate).
MATERIALS AND METHODS

Study Design and Project Participants

The type of study was controlled randomized study on fifty five infertile couples undergoing ICSI procedure at fertility center in Al-Sadder medical city in Al Najaf Al Ashraf under the supervision of Urology department in Kufa Medical College over one year. Informed consent was taken from all couples and steps of procedure were assessed and accepted by ethical committee of the department. Men partner from couple undergoing ICSI cycle, aged 20-45 years, without surgical history in (testis, vas deference and epididymis), no chromosomal anomalies, no chronic disease or medical illnesses and can give semen sample were eligible to enter the study. Full physical examination was done, weight and height measurements were performed. Blood sample was taken from all men by venipuncture and centrifuged at 3000 round for 10 minute. Serum samples were refrigerated under -20°C for later testing. Semen samples were taken from all participants after 3 days of abstinence for assessing semen parameters. The total sample (55 couples), were categorized into two classes according to the cause of infertility as male factor group and unexplained group. All patients characters are assessed in each group. Female partners of enrolled men were of normal weight, aged below 35 year and without identifiable cause of infertility. They were examined by obstetrician senior who performed a full examination and investigation including trans-vaginal ultrasound and hormonal analysis (FSH, LH, Prolactin , AMH, E2 and Progesterone) and placed them on short agonist protocol. Follow up of patients by trans-vaginal ultrasound and hormonal study was done until achieved appropriate response. HCG hormone was given to patients when ultrasound showed more than 3 follicle of size >18 mm.

Inclusion Criteria were include age of male from 20-45 years ,Mild to moderate male factor, Presence of functional testes, No chromosomal and chronic illnesses, Without surgical history in testis, vas deference and seminal vesicle, Cycles are yielded an follicle aspiration and embryo transfer, Testing and screening for hepatitis and human immune deficiency viruses should be negative.

Exclusion Criteria were include age of male <20>45 year, Sever male factor, Congenital anomalies of testes(undescended testis), History of treatment with chemotherapy or radiotherapy, and Males with female factor

Analysis of AMH

Blood samples were taken from infertile men before 10:00 a.m by venipuncture at day of ovum pick up and centrifuged 30 minute after collection at 3000 round for 10 minute to separate the serum, serum samples were aliquoted and frozen at -20°C for later AMH analysis. AMH was measured in serum by using kit from (Ansh lab, USA).

Statistical Analysis :Data were ordered, summarized and expressed by using two software programs: Microsoft Office Excel 2010 and Statistical Package for Social Sciences( SPSS version 20) . Categorical data were expressed as frequency and percentage. Continuous data were expressed as mean and standard error. Pearson s Chi square (X2) test and Fisher exact test were utilized to study the relation between any two categorical variables. T-test was performed to compare means between two groups. ANOVA was performed to find the mean differences between three groups or more. The relationship between two continuous variables was obtained by using correlation coefficient (r). A P value of less than or equal to 0.05 was considered significant

Results :The total sample was divided according to cause of infertility into male factor (56.4%) group and unexplained group (43.6%) .Age of patients and duration of infertility was found to be not
significantly differed among study groups. There was a significant difference in means of BMI (kg/m²) and AMH (ng/ml) between male factor group and unexplained group as shown in table (1).

Table (1): The demographic data and anti-mullerian hormone according to cause of infertility

| Study variable | Cause of infertility | Male factor (n=31) (Mean ± SE) | Unexplained (n=24) (Mean ± SE) | P value |
|---------------|----------------------|---------------------------------|---------------------------------|---------|
| Age           |                      | 34.45 ± 1.22                   | 34.88 ± 1.21                   | 0.811   |
| BMI           |                      | 29.32 ± 1.20                   | 24.13 ± 0.45                   | <0.001* |
| Duration      |                      | 6.97 ± 0.79                    | 8.29 ± 1.06                    | 0.312   |
| AMH           |                      | 2.36 ± 0.19                    | 7.29 ± 0.73                    | <0.001* |

The outcomes of ICSI in form of fertilization rate (%), cleavage rate (%) and total embryo were found to be not significantly differed among both groups while good quality embryo was found marginally lower in male factor group than unexplained group (table 2). Pregnancy rate was also found to be not significantly differed but the higher percentage of pregnancy (32.3%) occurred in male factor group compared to unexplained group (25%) (χ²=0.345, P=0.557) as shown in figure (1).

Table (2): ICSI outcomes in male factor group and unexplained group.

| Study variable | Cause of infertility | Male factor (n=31) (Mean ± SE) | Unexplained (n=24) (Mean ± SE) | P value |
|---------------|----------------------|---------------------------------|---------------------------------|---------|
| Fertilization rate (%) |          | 0.72 ± 0.05                   | 0.71 ± 0.04                    | 0.889   |
| Cleavage rate (%) |          | 0.96 ± 0.01                   | 0.94 ± 0.02                    | 0.451   |
| Good quality embryo |      | 5.70 ± 0.94                   | 3.70 ± 0.5                    | 0.053*  |
| Poor quality embryo |     | 1.08 ± 0.49                   | 1.67 ± 0.48                    | 0.401   |
| Total embryo   |          | 6.79 ± 1.02                   | 5.38 ± 0.65                    | 0.234   |

Serum AMH was found to be not significantly related to fertilization rate and total embryo but positively related to good quality embryo. There was negative correlation between serum AMH and poor quality embryo as shown in table (3).

Table (3): The correlation between AMH and study variables including fertilization rate (%), cleavage rate (%), good quality, poor quality and total embryo

| Study variable | R     | P value |
|---------------|-------|---------|
| AMH (ng/ml)   | 0.023 | 0.87    |
| Fertilization rate (%) | 0.293 | 0.03* |
| AMH (ng/ml)   | 0.293 | 0.03*   |
| Good quality embryo | -0.122 | 0.376 |
| AMH (ng/ml)   | -0.122 | 0.376 |
| Poor quality embryo | 0.186 | 0.173 |
| Total embryo  | 0.186 | 0.173   |

Discussion

In order to decrease the impact of female confounding factors, all female partners of infertile men in the study groups shared approximately the same demographic characteristics (normal BMI, age below 35 year, without identifiable cause of infertility and similar ovarian reserves). So any differences in
ICSI outcomes are related to men partners. In the present study, all patient characteristics (age, duration, AMH and ICSI outcomes) were compared in relation to cause of infertility either male factor or unexplained infertility. The current study was revealed that BMI was significantly high in male factor group compared to unexplained group. Similar observation was reported by other authors (Sallmen et al., 2006; Bakos et al., 2011; Hakonsen et al., 2011; Braga et al., 2012) while other studies demonstrated no association between BMI and male infertility (Duits et al., 2010; Relwan et al., 2011; Eskander et al., 2012), these might be due to fact that obesity could not affect sperm parameters. One of the important points in the present study is that serum AMH was significantly low in male factor group compared to unexplained group. In agreement with this result, other authors concluded that serum AMH was found to be significantly low in men with abnormal semen parameters in male factor group than men with normal semen parameters in unexplained group (Al-Qahtani et al., 2005; Appasamy et al., 2007). In contrast to these results, others found no association between male infertility and serum AMH (Tuttleman et al., 2009; El-Halawaty et al., 2011). Possible explanation for the association between AMH and semen parameters is that AMH is produced from Sertoli cells which are found in seminiferous tubules, the site of sperm production (Shaha, 2007). Therefore, the relation might exist. Regarding the outcomes of ICSI, we found that the fertilization rate, cleavage rate, embryo quality and total embryo not significantly differed among both group. The pregnancy rate was also found to be not significantly differed but the high percentage of pregnancy occurred among the male factor group than unexplained group which is in respect with other study on impact of semen parameters in both groups on ICSI outcomes and concluded no relation between semen quality and ICSI results (Alasmari et al., 2018). In contrast to these observations, other researchers suggested that sperm quality might affect embryo development from fertilization to blastocyst formation (Tesarik et al., 2002; Virro et al., 2004; Loutradi et al., 2006). In ICSI we used the better sperm for oocyte injection this is may be reason for why different sperm quality in both groups give approximately the same outcomes after ICSI procedure, another explanations: the female partner in unexplained group may have unidentifiable endometriosis or undiagnosed uterine problem, poor oocyte or sperm quality (Alasmari et al., 2018), a high percentage of spermatozoa in men with unexplained infertility were found to have a high level of sperm DNA damage which may affect outcome after ICSI process (Simon et al., 2013). Regarding the relation between serum AMH and ICSI outcome, we found no significant relation between serum AMH and fertilization rate while a significant positive correlation was found between AMH and embryo quality. Because there was a significant positive correlation between serum AMH and semen parameters, increase in rate of sperm aneuploidy and sperm DNA damage in men with poor semen parameters might affect ICSI outcome (Vegetti et al., 2000; Martin et al., 2003; Durak Aras et al., 2012; Sheikh et al., 2008). Zheng et al. (2018) demonstrated that sperm DNA fragmentation had a negative effect on embryo development in ICSI process. A previous study was conducted by Ahmadi and Ng. (1999) showed that sperm DNA damage could not affect fertilization step but affect subsequent embryo development. Only two studies were found about the relation between men serum AMH and ICSI outcomes and showed no effect of serum AMH on ICSI results (El-Halawaty et al., 2011; Zhang et al., 2011). In conclusion, this study demonstrated found positive correlation between male serum AMH and good quality of embryos also there are increment of the levels of anti-mullerian hormone in unexplained infertility group compared to male factor infertility group.

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