

The impact of paternal obesity on serum anti-müllerian hormone level and outcome of intracytoplasmic sperm injection in infertile couples

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Abstract

Objectives: The goal of our study was to investigate the effect of male body mass index on serum anti-mullerian hormone (AMH) and outcome of intracytoplasmic sperm injection (ICSI) (fertilization rate, cleavage rate, embryo quality, total embryos and pregnancy rate). **Patients and methods:** A prospective cohort study was carried on fifty-five infertile couples who encountered inclusion criteria. Blood samples were analysed for AMH. Semen samples were collected and examined according to World Health Organization, 1999. Weight and height measurements were performed and patients were divided by BMI into normal weight (BMI 18.5-24.9 Kg/m², n=21), overweight (BMI 25-29.9 Kg/m², n=22) and obese (BMI >30 Kg/m², n=12). **Results:** A negative correlation was found between BMI and serum level of AMH (r=-0.507, p=0.001). Fertilization rate, cleavage rate, total embryo and pregnancy rate were found to be not related to BMI while the good quality embryo was a significantly high in normal weight men compared to overweight and obese men (6.47±0.96, 3.77±0.66, 2.75±0.66). Sperm concentration was found significantly high in normal weight men compared to overweight and obese men (39.90±6.06 million /ml, 24.73±6.14 million/ml, 7.42±1.73 million /ml). Progressive sperm motility was also high in normal weight than overweight and obese men (47.67±4.53, 30.82±4.16, 15.67±4.01). No significant difference was found in sperm morphology among study groups. **Conclusion:** Our findings suggest that BMI may have a negative effect on the serum AMH level and semen parameters with no effect on ICSI outcomes except for good quality embryo.

Keywords: Paternal obesity, Serum, Anti-müllerian hormone, Intracytoplasmic .

INTRODUCTION

Infertility defined as a failure of the woman of reproductive age group to conceive after one year of frequent, unprotected regular vaginal intercourse with an absence of any obvious cause of infertility and should be offered additional clinical evaluation and investigation along with her husband with earlier referral to the specialist if her age above 36 year (NICE clinical guidelines, 2013). For couples who had no previous pregnancy, infertility defined as primary infertility while those difficult ones to achieve pregnancy with previous normal pregnancy child are defined as secondary infertility (Desai et al., 2011; WHO, 2013). According to European society of human reproduction and embryology (2014), 20-30% of infertility cases are due to male factor, 20-35% are due to female cause, 25-40% are due to combined factors and 10-20% no obvious cause is found. Most common causes of male infertility are varicocele, maldesended testes, genital tract obstruction, testicular failure, exposure to gonadotoxin, genetic disorder, infections, hormonal abnormalities, immunological condition and systemic illnesses (Esteves et al., 2012). According to WHO reports in 2016, 650 million and more than 1.9 billion people worldwide were obese and overweight respectively (Moussa et al., 2016). Obesity has been related to increase the risk of many medical chronic diseases including diabetes, hypertension, cardiovascular diseases, sleep apnea and infertility (Bieniek et al., 2016; Dubeux et al., 2016). The international classification of adult weight status classified weight into four categories; underweight (BMI < 18.5 Kg/m²), normal weight (BMI 18.5-24.9 Kg/m²), overweight (BMI 25-29.9 Kg/m²) and obese (BMI > 30 Kg/m²) (Moore et al., 2010). Many studies were performed to show the influence of female BMI on fertility and a negative impact of obesity on fertility demonstrated by many authors while few studies done to reveal the association between male infertility and obesity. Epidemiological researches concluded that increase risk of couple infertility with increasing BMI of male partner (Sallmen et

al., 2006; Ramalua et al.,2007; Nguyen et al., 2007). Antimullerian hormone is a dimeric glycoprotein, belongs to the transforming growth factor beta superfamily(Hample et al.,2011). AMH is produced by Sertoli cell and considered a specific marker for spermatogenesis(Al-Qahatni et al., 2005; Goulis et al.,2008;Matuszczak et al.,2013). AMH level has been disturbed by increasing men BMI. Few studies done to investigate the effect of increasing men BMI on serum AMH. Previous authors demonstrated a negative association between BMI and serum AMH (Pietilainen et al.,2012; Anderson et al., 2015) while other authors showed no association(Tuttelmann et al.,2009). The goal of present study was to increase the knowledge about the impact of paternal obesity on the serum AMH and outcomes of ICSI.

PATIENTS AND METHODS

The study was based on a cohort of 55 infertile couples undergoing ICSI procedure at the fertility center in Al-Sadder medical city in Al Najaf Al Ashraf under supervision of the Urology department in Kufa medical college over one year. Informed consent were taken from all couples and steps of procedure were assessed and accepted by the ethical committee of the department .Men partner from couple undergoing ICSI cycle , aged 20-45 years, without surgical history in the urogenital system, no chromosomal anomalies, no chronic medical diseases and can give semen sample were eligible to enter the study . Full physical examination were done , weight and height measurements were performed . Blood sample was taken from all patients by venipuncture and centrifuged at 3000 round for 10 minute. Serum samples were refrigerated under -80 for later testing. Semen samples were collected after three days of sexual abstinence and examined according to WHO,1999. Sperm preparation for ICSI cycle was done by swim up procedure which performed by layering culture medium over liquefied semen then motile spermatozoa swim up into the culture medium. The total samples(55 couples), were categorized into three classes according to BMI to the normal weight (BMI18.5-24.9Kg/m²n=21), overweight (BMI 25-29.9Kg/m²n=22), obese(BMI>30Kg/m²n=12) groups. Female partners of enrolled men were have normal weight, aged below35 years and without identifiable cause of infertility. They were examined by obstetrician senior who performed full examination and investigation including trans-vaginal ultrasound and hormonal analysis(FSH, LH ,Prolactin ,AMH, E2, Progesterone) and placed them on one of three stimulation protocols ;long agonist , short agonist and antagonist protocols. Follow up of patients by transvaginal ultrasound and hormonal study were done until achieved appropriate response .Human gonadotropin hormone(hCG) was given to patients and ovum pick up was performed 36 h after hCG injection when ultrasound showed more than 3 follicle of size >17 mm. Oocyte denudation was performed 2 hour after collection to remove cumulus cell and only mature oocyte(metaphase II) was inseminated by spermatozoa. Normally fertilized oocyte displayed two pronuclei and two polar bodies within 16-18 hour after insemination. Fertilization rate was estimated from number of normally fertilized oocyte divided by number of inseminated oocyte while cleavage rate was calculated from number of total embryo divided by number of fertilized oocyte. Good quality embryo usually reached four cell stage on day two and/or seven or eight cell stage on day three with less than 20% fragmentation otherwise considered poor quality embryo. Embryo transfer was performed on day three of luteal phase and 1-3 embryos were transferred. Pregnancy test was done 14 day after embryo transfer

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.

RESULT

In total 55 infertile men were involved in the study after excluding men with congenital abnormalities of testes, urogenital surgery and previous treatment with chemotherapy or radiotherapy. Demographic features of patients are described in table (1). The total sample was divided into three groups according to BMI, 38% of participants were normal weight (BMI 18.5-24.9 Kg/m²), 40% were overweight and 22% were obese(BMI>30 Kg/m²). There was significant difference in means of sperm concentration among study groups (39.90±6.06 million /ml, 24.73±6.14million/ml, 7.42±1.73 million /ml). Progressive sperm motility(%) also found to be significantly high in normal weight men compared to overweight and obese men (47.67±4.53, 30.82±4.16, 15.67±4.01). No significant difference found in means of sperm morphology(%) among three groups as described

in table 2. A negative correlation was found between BMI and serum AMH level ($r=-0.507$, $P<0.001$) as shown in figure 1. Regarding ICSI outcomes, there was no significant differences in fertilization rate, cleavage rate and total embryo but the good quality embryo found to be significantly high in normal weight men compared to overweight and obese men (6.47 ± 0.96 , 3.77 ± 0.66 , 2.75 ± 0.66) as revealed in table 3. The pregnancy rate found to be not significantly differed but a higher percentage of pregnancy occurred in the normal weight group than overweight and obese groups (33.3, 27.3%, 25% respectively) (figure 2).

Table 1: distribution of patients according to study variables

Study variables	N	(Mean \pm SE)
Age	55	(34.64 \pm 0.8)
Duration	55	(7.55 \pm 0.63)
BMI	55	(27.06 \pm 0.73)
AMH	55	(4.51 \pm 0.47)
Sperm concentration(million/ml)	55	(26.75 \pm 3.7)
Sperm morphology(%)	55	(35.55 \pm 2.7)
Sperm motility(%)	55	(33.95 \pm 2.98)
Fertilization rate(%)	55	(0.71 \pm 0.03)
Cleavage rate(%)	55	(0.95 \pm 0.01)
Good quality embryo	55	(4.58 \pm 0.5)
Poor quality embryo	55	(1.41 \pm 0.34)
Total embryo	55	(6.0 \pm 0.57)

Table 2: The effect of BMI on sperm concentration, progressive sperm motility and sperm morphology

Study variable	Body Mass Index			P value
	Normal (n=21)	Overweight (n=22)	Obese (n=12)	
	(Mean \pm SE)	(Mean \pm SE)	(Mean \pm SE)	
Sperm concentration (million/ml)	39.90 \pm 6.06	24.73 \pm 6.14	7.42 \pm 1.73	0.003*
Sperm morphology (%)	43.29 \pm 5.21	30.55 \pm 4.13	31.17 \pm 4.21	0.097
Sperm motility (%)	47.67 \pm 4.53	30.82 \pm 4.16	15.67 \pm 4.01	<0.001*

Table 3: The effect of BMI on ICSI outcomes.

Study variable	Body Mass Index			P value
	Normal (n=21)	Overweight (n=22)	Obese (n=12)	
	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	
Fertilization rate (%)	0.77 ± 0.04	0.70 ± 0.05	0.64 ± 0.07	0.33
Cleavage rate (%)	0.97 ± 0.01	0.94 ± 0.02	0.93 ± 0.04	0.466
Good quality embryo	6.47 ± 0.96	3.77 ± 0.66	2.75 ± 0.66	0.009*
Poor quality embryo	0.61 ± 0.25	1.63 ± 0.54	2.41 ± 1.13	0.136
Total embryo	7.09 ± 0.93	5.40 ± 0.92	5.16 ± 1.24	0.336

Figure 1: Shows the association between AMH (ng/ml) and BMI (kg/m²) among study patients. There was a significant negative association between BMI and serum AMH. ($r = -0.507$, $P < 0.001^*$), $Y = 13.21 + (-0.321 X)$.

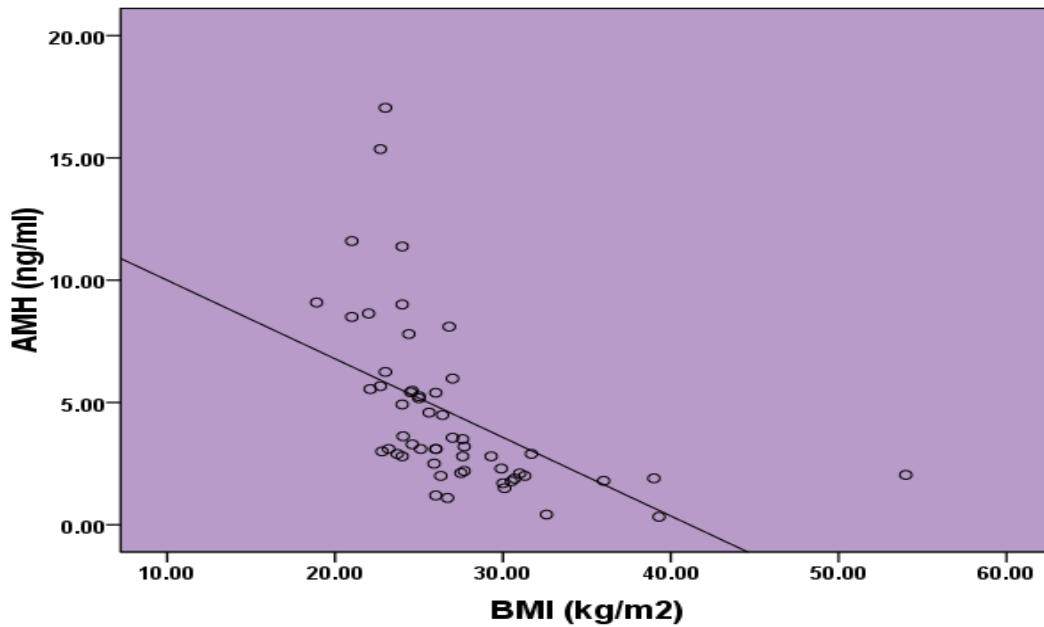


Figure 1: The correlation between AMH (ng/ml) and BMI (kg/m²) among study patients

Figure 2: shows the association between BMI and pregnancy rate among study patients. There was not significant association between BMI and pregnancy rate among study patients but the higher percentage of patients with normal weight (33.3%) get pregnancy ($X^2=0.316$, $P=0.854$) compared to that of overweight and obese men

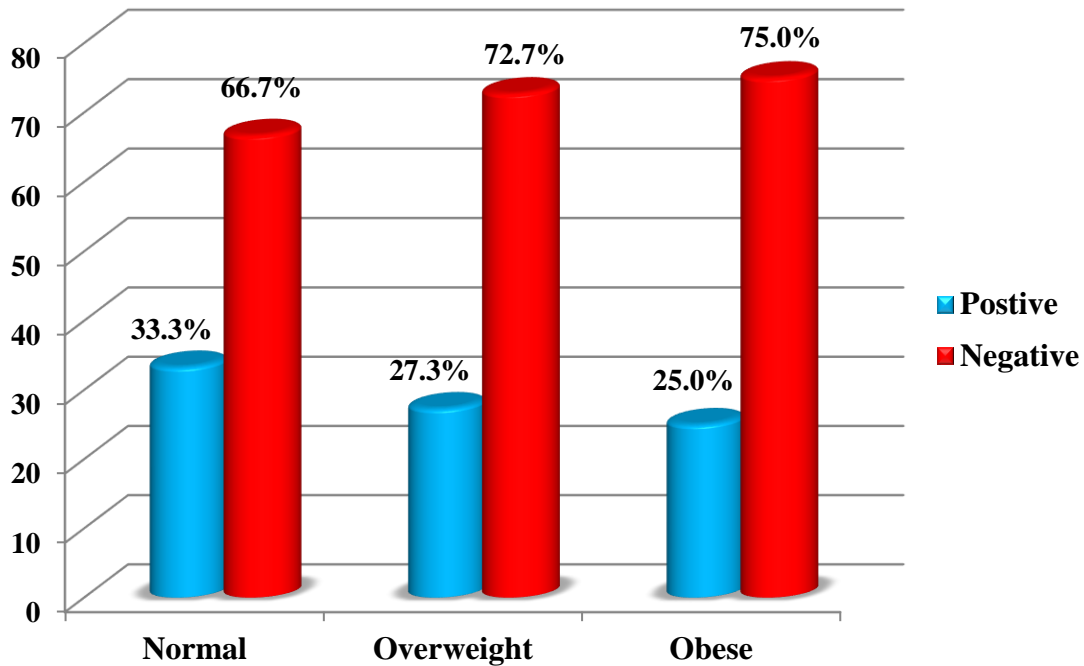


Figure 2: The association between BMI and pregnancy rate among study patients

DISCUSSION

Obesity is considered now as an epidemic dilemma that is rapidly increasing in developed and developing countries. The impact of obesity not only related to causing many chronic medical illnesses but also has been associated with impairment of reproductive functions (Dubeux et al., 2016; Bieniek et al., 2016). Potential negative effects of high men BMI on fertility has not been viewed to the same extent of research of female obesity. 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 years, without identifiable cause of infertility and similar ovarian reserves). So any differences in ICSI outcomes is related to men partners. In the present study, all patient characteristics (age, semen quality, AMH, ICSI outcomes) were compared between BMI categories. The results of our study indicate that BMI is an important element affecting semen parameters. Although semen parameters were varied in each BMI category, we found that men BMI negatively associated with semen parameters. The findings showed that obese and overweight men had significantly lower sperm concentration and decreased sperm motility than normal weight men with no association between BMI and normal sperm morphology. Many previous researches demonstrated the same results (Carreau et al., 2002; Hammoud et al., 2008; Martini et al., 2010; Macdonald et al., 2013; Belloc et al., 2014; Anderson et al., 2015) while other researchers demonstrated no association between BMI and any sperm parameters (Dutis et al., 2010; Relwani et al., 2011; Eskander et al., 2012). Obesity has been negatively affect semen parameters by many ways: disturbing hypothalamic-pituitary-gonadal axis (HPG), impair Sertoli and Leydig cell functions, elevated scrotal temperature and decrease sex hormone release (Sallmen et al., 2006; Ramlua-Hansen et al., 2007; Nguyen et al., 2007). Obese men tend to have low SHBG and inhibin B level with high estrogen level (Jensen et al., 2004; MacDonald et al., 2010). The most important point in the present study is the association between men BMI and serum AMH level, our results demonstrated that serum AMH decrease with increasing men BMI. (Pietilainen et al., 2012; Anderson et al., 2015, Anderson, 2017) showed that obese men have low serum AMH level compared to the normal weight men. Hakonsen et al., (2011) revealed that weight loss will increase AMH, testosterone and SHBG level in obese men. Other authors (Tuttelmann et al., 2009) demonstrated no association between BMI and serum AMH. The effect of obesity on men serum AMH may be possible reflected the direct negative impact of adiposity on Sertoli cell function (Anderson et al., 2015). Regarding ICSI outcomes among BMI groups, we found no significant difference in fertilization rate, cleavage rate, poor quality embryo among BMI

categories .In agreement with this observations , several researchers demonstrated no effect of increased BMI on ICSI outcomes(Keltz et al.,2010 Bakos et al.,2011; Petersen et al .,2013;Thomsen et al.,2014) ,while other studies concluded that increasing men BMI negatively influence the outcomes after ICSI cycle(Bakos et al.,2011; Hwang et al.,2011; Colaci et al.,2012; Merhi et al.,2013). Anifandis et al.,(2013) showed that increasing BMI among infertile men undergoing ICSI procedure adversely affect cleavage rate and quality of embryo. The data of the present study revealed that good quality embryos found to be significantly high in normal weight men than overweight and obese men this in respect with other study(Anifandis et al .,2013; Yang et al .,2016).Thomsen et al., (2014) found no association between BMI and embryo quality. Furthermore the pregnancy rate found to be not significantly differed among study patients but a higher percentage of pregnancy occurred in normal weight men compared to overweight and obese men. Poor semen parameters in overweight and obese men might be the main reason for decrement in good quality embryo. Loutradi et al.,(2006) demonstrated that embryo quality could be related to semen parameters. Other authors revealed that sperm quality affect ICSI outcomes from fertilization to blastocyst formation(Tesarik et al., 2002; Virro et al .,2004). Previous studies demonstrated that high BMI might induce alteration in hormones that might affect sperm DNA integrity which affect embryo quality(Chavarro et al.,2010; Zheng et al., 2018).Other authors (Anifandis et al.,2013) concluded that male obesity affect embryo quality by inducing alteration in sperm DNA integrity not by alteration of semen parameters.

CONCLUSION

Our results indicate that BMI may have negative influence on semen parameters and serum AMH. An increase BMI of male partner of infertile couples has negative effect on good quality embryo. Further large sample studies are needed to show the causes of low serum AMH level in infertile overweight and obese men and to reinforced the findings obtained by current study.

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