ORIGINAL ARTICLE

Effect of Body Mass Index on Intracytoplasmic Sperm Injection Outcomes

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ABSTRACT

Objective: To determine the effects of increased BMI on ICSI outcomes.
Study Design: It was an analytical cross sectional study.
Place and Duration of Study: Islamabad Clinic Serving Infertile Couples, Islamabad, Pakistan. Duration of the study was one year, from January 2013 to December 2013.
Materials and Methods: Total of 150 females were classified into three groups on the basis of BMI. Those with BMI ranging from 18.5 - 24.9 kg/m2 /were placed in normal group [n=50], those with BMI ranging from 25 - 29.9 kg/m2/were placed in overweight group [n=50] and those with BMI ranging more than 30kg/ m2 were placed in obese group [n=50]. Serum levels of FSH, LH, Te, TSH, T3 and T4 were done in all three groups and their effects on oocyte and embryo quality were observed during controlled ovarian stimulation and after ICSI procedure.
Results: Obese group had significantly decreased ICSI outcomes due to increased levels of testosterone (p value< 0.05).
Conclusion: Increased BMI leads to decreased ICSI outcomes.

Key words: *Obesity, Oocyte Quality, Embryo Quality, ICSI Outcomes, Infertility.*

Introduction

Infertility can be defined as inability to conceive after 12 or more months of regular unprotected sexual intercourse.[1] Pakistan is among the currently most populated countries of the world, and has a population growth rate of around 2%, it also has high rate of infertility (21.9%) amongst which 3.5% is primary and 18.4% is secondary.[2] The causes of female infertility relates mostly to the hypothalamus, pituitary gland, ovaries, the fallopian tubes, body of the uterus, cervix and the vagina.[3] The fecundity of females is sensitive to body weight. Girls require a particular threshold of body fat to enter puberty.[4]However, extreme body fat has adverse effects on female fecundity.[5] These effects present at several levels which include disturbed levels of gonadotropins, anovulation, disturbed steroid production, decreased conception rates, increased abortion rates and increased risk of other complications in pregnancy including hypertension, premature birth and increased frequency of fetal anomalies.[6,7,8] Adipose tissue is a fundamental site for synthesis and breakdown of steroids. It is also the site where androgens are converted to estrogens by activity of aromatase enzyme; estradiol is converted to estrone and dihydroepiandrosterone (DHEA) to androstenediol by 17b-hydroxysteroid dehydrogenase activity.[9] This leads to increased steroid levels in obese females which alters transmission of androgens and estrogens to their respective sites.[10] Serum concentration of sex hormone binding globulin (SHBG) is decreased in obese women. Decreased levels of SHBG lead to elevated levels of testosterone, dihydrotestosterone and androstenediol. This relative hyperandrogenaemia seen in obese women may result in decreased ovarian activity causing menstrual irregularities and oligoovulation or anovulation.[6, 11] The increase prevalence of obesity and its adverse effects on fertility in overweight and obese women has made assisted reproduction technology (ART), such as Intracytoplasmic Sperm Injection (ICSI) as the opted treatment amongst these groups. During the ICSI procedure high doses of exogenous gonadotropins are required for follicular growth and collected oocytes are fertilized in vitro by sperm injection. Developing embryo, 2 to 5 days later is transferred into the female uterus. Pregnancy is confirmed by increased serum beta hCG levels.[12] Before the beginning of ICSI procedure, a rapid evaluation of the retrieved oocyte for maturity is done. The quality is assessed by presence of degenerative changes in the cytoplasm, polar body (PB) or zona pellucid.[13] Embryo quality (day -3 scoring) is classified on the presence of number of blastomeres along with the cleaving status of the embryo, it provides information of embryo health

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and its chances of resulting into pregnancy in infertile couples as shown in Fig 1.

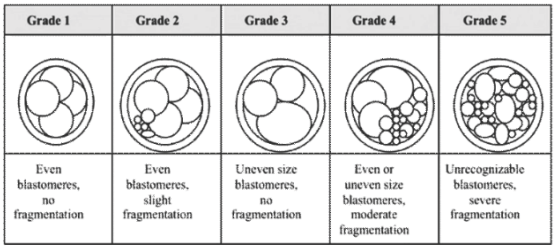

Fig 1: This image shows Embryo grading (day -3 scoring) Classification according to Veeck.[14]

In the present study association between increase body mass index and serum levels of reproductive hormones (FSH, LH, Te) and thyroid profile on oocyte quality and intracytoplasmic injection (ICSI) outcome in infertile female attending infertility clinic was done.

Materials and Methods

The study was conducted at Islamabad clinic serving infertile couples, Islamabad Pakistan. The duration of the study was one year, from January 2013 to December 2013. It was an analytical (Cross sectional) study. A total of 150 female subjects were included in the study. The subjects were divided into three major groups according to BMI classified by WHO. BMI ranges from 18.5 - 24.9 kg/m2were placed in normal group [n=50], BMI ranges from 25 - 29.9 kg/m2 were placed in Overweight group [n=50] and BMI ranges more than 30kg/ m2 were placed in Obese group [n=50]. Probability (systametic) sampling procedure was used. Sample size was calculated by open Epi sample size calculation for cross sectional, cohort and clinical trials.[15]Consent from subjects and permission from institutional review committee (faculty of health & medical sciences) was obtained for the study. Normal, overweight and obese infertile females between 20-39 years of age having ovarian cause of infertility, females with tubal blockage as cause of infertility were included in the study. Females having uterine and cervical causes of infertility were not included in the study. The variables included in the study were BMI, serum levels of FSH, LH, Testosterone, TSH, T3, and T4. The blood sample was taken on day-3 of the menstrual cycle for hormonal assay of serum FSH, LH, Testosterone, T3, T4, and TSH. Analysis was done by ARCHITECT i technology by using Chemiluminescent Microparticle Immunoassay (CMIA). Down regulation was done by giving subcutaneous Gonadotrophin-Releasing Hormone (GnRH) agonist depot preparation (Suprefact 0.1mg; Sanofi Aventis. Guildford, UK). Ovarian stimulation was done by giving subcutaneous administration of recombinant FSH 50-IU preparation (Puregon; NV Organon, Oss, The Netherlands). The ovarian follicular response was monitored by trans-vaginal ultrasound three to four days after the commencement of the ovarian stimulation. Monitoring was done on alternate days. When the size of the leading follicles on ultrasound was more than 18mm in diameter, 10,000 IU of hCG (Ovitrelle; Serono, Rome, Italy) was administered intra-muscularly. Ovarian retrieval was done 351/2 – 36 hours after hCG injection using the vaginal ultrasound technique under general anesthesia. Half an hour after egg collection the eggs were denuded prior to microinjection using Hyalurunidase (Hyase; Vitrolife) and then rinsed several times in droplets of culture medium and were observed under the microscope. During this process oocyte quality was observed and those oocyte that had excruded the first polar body (metaphase II stage) were considered mature eggs of good quality.ICSI was carried out on all mature eggs. Fertilization was confirmed 16-18 hours after ICSI procedure. Cleavage was confirmed after another 24 hours of in vitro culture. All embryos graded according to Veeck classification (Fig 1) prior to embryo transfer on day 3 of egg collection. Good quality embryos were transfered by using Sims-Wallace Embryo Replacement Catheter (SIMS – Portex Limited, Hythe Kent, UK) of blastocyst stage on day-3 of egg collection under ultrasound guidance.Progesterone (Cyclogest ®400 vaginal pessaries; Shire UK) were given until Pregnancy Test was done and continued for 12 weeks if subject was pregnant. Twenty one (21) subjects out of 150 were excluded from the study which included eleven (11) from overweight group and ten (10) from obese group before embryo transfer. These subjects suffered from Ovarian Hyperstimulation Syndrome (OHSS), an iatrogenic complication for ovarian stimulation by intracytoplasmic sperm injection procedure following gonadotropins. SPSS version 17 was used for data analysis. Mean ± SD were calculated for body weight. Median ± SD were

calculated for hormones. Stratification was done with regards to BMI. One way ANOVA test was used. P value of < 0.05 was considered significant.

Results

Effect of increased BMI on serum hormone levels in normal, overweight and obese groups is shown in the table I. Testosterone was significantly raised in obese group (*P<0.001). A Post Hoc Tukey test revealed that serum testosterone levels were significantly raised in obese group than normal and overweight groups as shown in table II. The embryo quality was measured by embryo grading (blastomere) and total number of blastocyst formed as shown in table III and IV in normal overweight and obese groups. The effect of increased BMI on ICSI outcomes in normal, overweight and obese groups is shown in table V. The results show statistically significant reduced ICSI outcomes (Pregnancy Test) in obese group (P<0.001).

Table I: The effects of increased BMI on serum hormone levels in normal, overweight and obese groups (n=50)

Hormones	Body Mass Index			P-value
	Normal Median±SD	**Over Weight** Median±SD	**Obese** Median±SD	
Testosterone ng/mL	0.7 ± 0.9	9.5 ± 13.4	22.4 ± 30.2	0.00 *

*P ≤ 0.05 considered significant

Table II: Post hock for serum testosterone levels amongst normal, over weight and obese groups

Body Mass Index	N	Subset for alpha = 0.05		
		1	2	3
Normal	50	.7438		
Overweight	39		9.5569	
Obese	40			22.4470

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 42.468.
b. The group sizes are unequal. The harmonic mean of the group sizes is used.

Table III: The effects of increased BMI on the embryo quality (Blastomere) in normal, overweight and obese groups (n=50)

Embryo Quality (Blastomere)	Body Mass Index			P-value
	Normal Mean±SD	**Over weight** Mean±SD	**Obese** Mean±SD	
Grade-I	1.84±0.8	1.96±0.8	1.92±0.9	0.7
Grade-2	1.12±0.4	1.36±0.6	1.24±0.5	0.09
Grade-3	1.0±0.0	1.04±0.19	1.02±0.14	0.3

P ≤0.05 considered significant

Table IV: The effects of increased BMI on embryo quality (Blastocyst) in normal, overweight and obese groups (n=50)

Embryo Quality (Blastocyst)	Body Mass Index			P-value
	Normal Mean±SD	**Over weight** Mean±SD	**Obese** Mean±SD	
Blastocyst	1.5±0.7	1.5±0.8	1.5±0.7	0.8

P ≤0.05 considered significant

Table V: The effect of increased BMI on ICSI outcomes in normal, overweight and obese groups (n= 50)

ICSI Outcomes	Body Mass Index				P-value
	Normal n=50	**Over Weight n=39**	**Obese n=40**	**Total n=129**	
Pregnancy Test (Positive)	30 (60%)	3 (7.69%)	8 (20%)	41 (31.7%)	0.00*
Pregnancy Test (Negative)	20 (40%)	36 (92.31%)	32 (80%)	88 (68.3%)	

*P ≤0.05 considered significant.

Discussion

In our study, it was observed that females belonging to obese group were associated with lower ICSI outcome as compare to females in normal or overweight group. A significant increase in serum testosterone levels was also observed in obese group of population. Our study was comparable with the study conducted by Fedorcsa´k et al[16] and, Loveland et al[17], Wang et al[18], Winter et al[19], and Maheshwari et al[20] who documented significant association between excess body weight and negative outcome in ICSI procedures, thereby implicating deleterious effect of increased BMI on ICSI outcomes. In contrast, Beydoan et al[21], Sathya et al[22], Matalliotalis et al[23] reported that increased BMI had no adverse effects on ICSI/IVF outcomes. A significant increase in serum testosterone levels was observed in obese group as

compared to normal and overweight group. The fact that our results showed increase in serum testosterone levels may be related to overweight and obesity which are associated with multiple metabolic changes. These include peripheral aromatization of estrogens, decreased levels of SHBG resulting in high levels of free estradiol and testosterone, and increased insulin levels that can enhance ovarian production of androgens lined by factors in our study population and limitations in other reports. In addition, it also indicates that high serum Testosterone levels (hyperandrogenaemia) affect the process of folliculogenesis resulting in ovarian dysfunction. Our study results were comparable with Metwally et al[7], who reported in his study that hyperandrogenaemia resulted in granulosa cell apoptosis, while peripheral conversion of androgens to estrogen in adipose tissue inhibited gonadotropin secretion. Another study conducted by Sharique et al[24] showed a statistically significant increase in mean serum testosterone levels in obese patients in comparison with control. Sowers et al[25] reported that women in the BMI category >30kg/m2 had serum testosterone concentrations that were 50–70pg/ml higher than the women in the BMI category ≤ 22kg/m2. Nardo et al[26] reported that pre pregnancy serum testosterone concentrations and BMI didn't have a statistically significant relationship with pregnancy outcomes. The reason for these findings could be the inclusion of large sample size, and different age groups of females included ranging from 18-44 years.

Conclusion

According to our study increased BMI effects serum testosterone level that result in low pregnancy rate (ICSI outcomes) in obese women. The results of the present study suggest that decreasing weight before opting for ICSI procedure would increase the chances of successful ICSI outcomes by improving oocyte quality which is affected by high levels of testosterone and reduces the chance of miscarriages. So, losing weight before ICSI treatment increases the chances of pregnancy.

Recommendations

Future recommendations regarding this study is that larger sample size, male cause of infertility, serum prolactin levels and uterine cause of infertility should also be included.

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