Original Article

Effect of Obesity on Ovarian Reserve Parameters in mid-reproductive age Women.

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Summary:

Background:The initiation and maintenance of reproductive functions are related to an optimal body weight in women. Body weight affectthe ovarian reserve which is basically an estimate of how many oocytes (eggs) are left in the ovaries.

Objective: To study the relationship between obesity and serum and ultrasound markers of ovarian reserve in mid-reproductive age women (21- 35 years old).

Patients and method:Twenty participants ("obese") had a body mass index (BMI) of 30 to 35 Kg/m2 and another 20 participants ("non-obese") had a BMI20-29 kg/m2. The obese women had a mean age of 27.9 years and the non-obese women had a mean age of29.5 years. Blood samples were collected from all participants, anthropometric measurements were calculated, and transvaginal ultrasonography was performed to measure the antral follicle count (AFC) during the early follicular phase. The blood samples were assayed for antimüllerian hormone (AMH), follicle-stimulating hormone (FSH) and estradiol (E2).

Results: Therewas no significant difference between the two groups regarding ovarian reserve markers and there is no significant correlation between these markers and BMI, except forserum E2 in the obese group.

Conclusion: Obesity has no effect on the levels of serum FSH, AMH, orAFCindicating that obesity is unlikely to affect ovarian reserve in the mid-reproductive age group.

Keywords: Antimüllerian hormone (AMH),follicle stimulating Hormone (FSH), body mass index (BMI),obesity,antralfollicleCount (AFC), ovarian reserve.

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Introduction:

The initiation and maintenance of reproductive functions are related to an optimal body weight in women. Underweight (BMI under 19 kg/m2), as well as overweight (BMI over 25 kg/m2) and obesity (BMI over 30 kg/m2) are associated with an increased risk of certain disorders (1). In addition to conditions such as diabetes mellitus, hypertension, cardiovascular disease, pancreatitis, and musculoskeletal diseases, obese women are more likely to experience reproductive problems (2,3), which include menstrual disorders, infertility, and maternal complications during pregnancy(4,5) . Overweight women, as distinct from obese women, are known to be at higher risk of menstrual dysfunction and anovulation. The mechanisms by which obesity causes or exacerbates subfertility are manifold, one suggested theory that Hyperandrogenaemia results in granulosa cell apoptosis, while peripheral conversion of androgens to estrogen in adipose tissue inhibits gonadotrophinsecretion(6),or possibly due to altered secretion of pulsatile GnRH. (7). Obesity is also associated with polycystic ovary syndrome (PCOS) which is a heterogeneous condition characterized by oligo or anovulation, hyperandrogenism, menstrual irregularities and subfertility (8, 9). Overweight and obese subfertile women have a reduced probability of successful fertility treatment and their pregnancies are associated with more complications and higher costs (10). Weight loss regularizes menstrual cycles and increases the chance of spontaneous ovulation and conception in anovulatory overweight and obese women. In women undergoing assisted reproductive technology being obese or overweight has been associated with a need for higher doses of gonadotropins, increased cycle cancellation rates, and fewer oocytes retrieved than in women of normal weight(11). Lower rates of embryo transfer, pregnancy, and live birth have also been reported in these women, and have higher miscarriage rates (12).

The term "ovarian reserve" refers to the quantity and quality of a woman's current reservoir of oocytes, and is closely associated with reproductive potential. It is an indirect measure of a woman's reproductive age (13). Over the past two decades, a number of tests of ovarian reserve have been used to determine follicle number and quality and to predict the outcome of assisted reproduction procedures (14). The woman's age and assays of serum FSH in the early follicular phase were among the earliest and most useful parameters used for evaluation of ovarian reserve (15, 16). Several ultrasound parameters have been used for evaluation of ovarian reserve, including ovarian volume(17, 18) and the antral follicle count, with varying degrees of reliability(19). Recently, serum

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antimüllerianhormone (AMH) levels have been introduced as a novel measure of ovarian reserve. AMH is a product of the granulosa cells in preantral and antral follicles (20) Serum AMH levels decline with age and are correlated with the number of antral follicles and the ovarian response to hyperstimulation(21).Few studies have evaluated the effect of obesity on ovarian reserve. The present study was conducted to examine the effect of obesity on ovarian reserve in women in the mid reproductiveage group. We assessed the effect of obesity on accepted markers of ovarian reserve, specifically levels of basal FSH, E2 and AMH, as well as the ultrasound marker of AFC.

Patients and methods:

This study was conducted in the fertility center of Al-SaderMedical City, in Al Najaf provincefromDecember 2010 to March 2011. All participating women gave written informed consent before beginning the study. We performed a cross-sectional comparative study of two age-matched groups of 20 participants (group A, obese women) had a BMI of 30- 35 kg/m2, with mean age 27.9 years, and the other 20 participants (group B, non-obese women) BMI of 20 -29 Kg /m2 with mean age of 29.5 years ,these serve as a control group. Blood samples were collected from all participants, and transvaginal ultrasonography was performed to measure the antral follicle count (AFC) during the early follicular phase. The blood samples were assayed for antimüllerian hormone (AMH), follicle-stimulating hormone (FSH) and estradiol (E2). Thyroid function test and serum testosterone as well as dehydroepiandrosterone serum levels were assessed as well .The women were seeking treatment for infertility because of tubal factor proved by hysterosalpingiography or laparoscopy. To meet the inclusion criteria, women had to be in the mid –reproductive age(20- 35 years) according to Stages of Reproductive Aging Workshop (STRAW)(22), with an intact uterus and ovaries and to have a regular menstrual cycles for the previous three months ,normal thyroid function and no evidence of hyperandrogenism by examination or hormonal assessment. Exclusion criteria were: current use of hormones or drugs that may affect ovarian function, smoking, pregnancy, lactation, previous ovarian surgery, clinical or ultrasound criteria suggesting polycystic ovarian syndrome or endometriosis, or any medical condition that might affect ovarian function. All participating women underwent a comprehensive history and thorough physical examination, calculation of BMI, assays of serum FSH, E2 and AMH, and had a transvaginal ultrasound examination for assessment of AFC. For calculation of BMI, height and weight were measured using the same scale for all participants. . BMI was determined by the ratio of weight in Kg divided by the height squared in metric units. Blood samples were withdrawn from the antecubital vein on cycle day 2, 3 of the menstrual cycle in all women. All samples were centrifuged at 2000 g for 15 minutes. Serum was separated and stored at –20 °C until assayed. Measurement of serum FSH was performed using Mini VIDAS method (bioMérieux® France). Inter-assay Coefficient of Variance %(CV %) 4.7; Intra-assay CV% 5.9, lower limit of detection ≤ 0.1mIU/ml within 95% probability. For E2 the kit we used(bioMérieux® France) with Mini VIDAS technique Inter-assay CV% 4.6; Intra-assay CV% 3.2.Lower limit of detection9 pg/ml Within 95% probability.Serum levels of AMH were determined by enzyme-linked immunosorbent assays(ELISA)using (Bckmancoulterinc,USA) kit ., the assays were done according to the manufacturer's instructions. The detection limits of this assay were 0.08ng/mL within 95% probability, and its intra-assay and inter-assay coefficients of variation were 5.6% and 4.5% respectively.Transvaginal ultrasound was performed during the early follicular phase (cycle day 2or 3), by means of a transvaginal ultrasound scanner (Philips 11*E), with a 5 MHz probe. In each ovary, the total number of small follicles (2–8 mm) was counted. The total follicle count was the sum of the follicle counts in each ovary.

Statistical analysis: descriptive statistics were expressed as mean and standard deviation. Student>s t-test was used to compare groups. Significant relationships between study parameters were evaluated by Pearson'scorrelationcoefficien t.P- Values< 0.05 were considered to be significant. Statistical analysis was performed using SPSS version 17.

Results:

The 20 women in group A (obese women) had a mean BMI of 32.45 kg/m2, with a range of 30 to 35 kg/m2, and the 20 non-obese women(group B) had a mean BMI of 24.9 kg/m2, with a range of 20 to 29 kg/m2. The mean age in the obese group was 27.9 years; with a range of 22 to 35 years. The mean age in the non-obese group was 29.5 years; with a range of 21 to 35 years. The mean BMI in the obese group (32.45 ± 1.57) was significantly higher than that of the non-obese group (24.9 ± 2.57) ($P < 0.05$). There was no significant difference between the two groups regarding age, serum levels of AMH or FSH, E2, or AFC. These data are shown in the Table (1). There was no significant correlation between BMI and serum AMH, serum FSH and AFC in both groups; but significant positive correlation at $p<0.05$ level was found between BMI and serum E2 in group A only, these results are shown in table (2).

Table (1): The studied parameters in the two groups.

Parameter	Group A (obese women)	Group B (non- obese women)	p- value
Age(years)	27.9 ± 4.29	29.50 ± 4.76	NS
BMI(Kg/m2)	32.45 ± 1.57	24.9 ± 2.57	P <0.05
AMH(ng/ml)	3.06 ± 1.49	2. 83 ± 3.51	NS
FSH(mIU/ml)	5.56 ± 2.12	5 .63 ± 2.53	NS
E2(Pg/ml)	40.64 ± 16.16	40.11 ± 19.53	NS
AFC	7.5 ± 1.61	7.3 ± 3.61	NS

The values are expressed as mean ± SD.

Table (2): Correlation of BMI with FSH, E2, AMH and AFC in group A and B.

Parameter	Group A	Group B
FSH(mIU/ml)	0.359	0.387
E2(Pg/ml)	0.469*	0.260
AMH(ng/ml)	_0.244	0.075
AFC	0.198	0.098

*p<0.05

Discussion:

Obesity is an increasingly prevalent health hazard andcauses many disorders of female reproduction (23, 24). In fact, overweight women have a higher incidence of menstrualdysfunction, anovulation, and infertility than other womenof reproductive age (25). Even though altered pulsatilegonadotropin secretion is a well-defined mechanism inobese patients (26).This study performed in (20) obeseand (20) non-obese women with normal menstrual cycles who were referred to fertility centerbecauseoftubal factor infertility. Our aim was to examine the possible effects of body mass on some ovarian reserve markers namely FSH, E2,AMH plasma levels and the number of ovarian follicles in the early follicular phase.The women included were normallyovulatingobese and non-obese, with regular menstrualcycles and with neither clinical nor hormonal signs of hyperandrogenism in their mid-reproductive age.

Several studies have suggested a negative effect of obesity on parameters of ovarian reserve. De Pergola and his coworkerssuggestedthat overweight and obese fertile women, in comparison with women of normal weight, have lower serum levels of FSH, LH, inhibin B, and estradiol in the early follicular phase, with a possible direct inhibitory effect of body masson gonadotropin and estradiol production, independent of age (27). The difference between their study and our findings may be attributed to selection ofBMIof the control group which was normal (BMI<25 Kg/m2), compared to our control group which included BMI> 25 Kg/m2. Other investigators reported lower levels of AMH in obese women compared with normal weight women in the late reproductive age(28). However,these studies documented that obesity had no effect onovarian follicle count. They suggested that lower levels of AMH in obese late reproductive age women result from physiologic processes other than decreased ovarian reserve (27).Our results showed that there are no significant differences in serum levels of FSH, E2,AMH, and AFCbetween obese and non-obese women. There was no significantcorrelation between BMI and the serum or ultrasound markers of ovarian reserve. Accordingly, we are suggesting that obesity may have limited effect on ovarian reserve in mid reproductive age women.The fact that our results showed no effect of obesity on AMH levels, contrary to other studies, may be related to factors in our study population and limitations in other reports. Our group of obese women was limited to women with a BMI between 30 and 35 kg/m2. We did not include morbidly obese patients because we thought that this specific group of women may have a different endocrine profile that may not apply to women with lesser obesity.In a study of women with polycystic ovary syndrome byPignyand his teamfound that AMH levels were lower in obese than non-obese women, but the difference was not statistically significant (29). Another study suggested no correlation between BMI and AMH in women with polycystic ovary syndrome and control subjects (30).These data may support our findings. We did not find an effect of obesity on AFC, which has been suggested by others (27, 28). This supports our impression of a limited effect of obesity on ovarian reserve.hiscollequeshowed that ovarian volume decreases with an increase in the BMI, indicating the possible decrease in fertility with an increase in a woman>s weight, their study group included normal weight and overweight and includes higher age study group than ours (31). This decrease may be due to age effect rather than BMI. They didn't find any correlation between BMI and AFC, which is in consistent with our study results.Inastudy conducted in Tehran (32) where they included 115 fertile women of different age group 25- 45 they found that BMI had moderate positive correlation with FSH moderate negative correlation with estradiol and AFC, but after adjustment of age, BMI as an independent factor had no effect on ovarian reserve markers, a finding which supports our results.

The significant positive correlationof BMI with estrogen in obese women may be attributed to the contribution for estrogen from the conversion of androgens to estrogens by aromatase in adipose tissue, or may be due to subtle undetected lack of insulin that increases the blood cholesterol concentration. These effects are probably caused mainly by changes in the degree of activation of specificenzymes responsible for the metabolism of lipid substances including cholesterol which is the precursor of estrogen (33). The difference between our finding and that found by other researchers may attributed to ethinec difference or life style factors(34, 35).The negative correlation between BMI and AMH have been confirmed by Pingy et al but it doesn't prove to be significant(29).

Conclusion:

Obesity doesn't have an effect on the selected parameters of ovarian reserve among our cohort of mid reproductive age women. However, this should be verified by larger studies with clear distinctions between normal, overweight, obese, and morbidly obese women, and between groups of different age groups.

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