Obesity and follicular fluid oxidative stress: Relationship to ICSI outcome

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Objective: To study the influence of female obesity on follicular fluid oxidative stress and to correlate it to intracytoplasmic sperm injection (ICSI) outcome.

Study design: Seventy-four normal females below the age of 40 undergoing ICSI for reason of male factor infertility were enrolled in the study. They were divided into 2 groups according to body mass index (BMI); Group I (non-obese) (n = 24, BMI < 25 Kg/m^2) and Group II (obese) (n = 50, BMI ≥ 25 Kg/m^2). Oxidative stress markers (MDA (malonaldehyde), NO2/NO3 (Nitrite/Nitrate) ratio, GSH (reduced glutathione) and GSH/GSSG (reduced glutathione/oxidized glutathione) ratio) were measured by high performance liquid chromatography (HPLC).

Results: Obese women had significantly higher mean follicular fluid MDA (P = 0.006) as well as NO2/NO3 ratio (P = 0.004). BMI strongly correlated to follicular fluid MDA (P = < 0.01). MDA showed strong positive correlation to NO2/NO3 ratio (P = 0.02). GSH and GSH/GSSG ratio showed a non-significant difference between the two groups (P = 0.14 and 0.67, respectively). Clinical pregnancy rate was significantly higher in the non-obese group (87%) compared to the obese (43%) (P = <0.01, OR:13; 95% CI 3.54–52). With binary logistic regression, MDA was found to be a good predictor of the occurrence of pregnancy (P = < 0.01). No significant differences were

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KEYWORDS
Obesity; Oxidative stress; Antioxidants; ICSI; Pregnancy rate

Abstract

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1. Introduction

Obesity has been linked to female morbidity. Its influence on female fertility is a field of much research. Although, obesity has been mostly linked to anovulation (1), still regularly menstruating obese women may suffer from poor reproductive potential (2). Endothelial oxidative stress, increased vascular endothelial cell production of NADPH oxidase, overactive mitochondria and excessive reactive oxygen species (ROS) production in oocytes and zygotes have been linked to female obesity (3,4). Follicular fluid is the environment surrounding the oocyte and influences oocyte quality and its interaction with sperm consequently affecting implantation and embryo development (5,6).

Overproduction of reactive oxygen and nitrogen species (RONS) has been linked to many stimuli among which is over consumption of dietary fats and carbohydrates (7,8). The mechanism by which lipotoxicity induces oxidative stress might be through the adverse effects of excess intracellular free fatty acids on mitochondrial membrane releasing ROS which are highly toxic to the cell. If not neutralized by antioxidant enzymes, ROS react with different cell molecules, proteins and lipids causing cell membrane and DNA damage. This occurs in many organs including female reproductive organs (9,10).

In addition, to oxidative stress (OS), another stress reaction to increased intracellular fat has been postulated which is the endoplasmic reticulum (ER) stress (11,12). Both ER stress and OS are inter connected and are able to enhance each other. Moreover, they are linked to inflammatory pathways that were found to be aggravated by obesity consequently causing cellular damage (13,14).

To what extent OS could influence the results of assisted reproductive techniques (ART) are an area of debate (9). Oxidative stress may affect ART outcome at different levels starting from oocytes, sperms and embryos up to the endometrium at the stage of implantation (10). In contrast, it has been postulated that some degree of OS should exist for pregnancy to be achieved through in vitro fertilization (IVF) (15). However, this should be balanced by adequate antioxidant response in order to ameliorate the harmful effects of excess pro-oxidants (10).

The aim of the current study is to assess the influence of female obesity on follicular fluid OS by measuring two OS markers; Malonaldehyde (MDA) and Nitrite/Nitrate (NO$_2$/NO$_3$) ratio; as well as two antioxidants; reduced glutathione (GSH) and reduced/oxidized glutathione (GSH/GSSG) ratio; and correlating their levels to ICSI outcome.

2. Materials and methods

This prospective cohort study was conducted at a private infertility center from July 2011 till August 2012. Seventy-four females undergoing ICSI were enrolled in the study. The indication for ICSI was male factor or unexplained infertility. Participants were divided according to BMI, (body mass index = Weight [kg]/Height [m$^2$]), into two groups: Group I (non-obese) ($n = 24$, BMI < 25 Kg/m$^2$) and Group II (obese) ($n = 50$, BMI ≥ 25 Kg/m$^2$).

Females below the age of 40 years, with regular menstrual cycles and normal hormonal profile were included in the study. Females having polycystic ovary syndrome (PCOS), ovarian hyperstimulation syndrome (OHSS), FSH ≥ 10 IU/L, tubal factor, endometriosis, endocrinal disorders and smokers were excluded from the study.

All patients received long protocol for controlled ovarian hyperstimulation (COH). Pituitary down regulation using gonadotropin releasing hormone (GnRH) agonist (Decapeptyl 0.1 mg, Ferring, Switzerland) was started from the luteal phase of the preceding cycle. With the start of menses, human menopausal gonadotropin (Menogon, Ferring, Switzerland or Merional, IBSA, Switzerland) was started as a daily dose of 150–300 IU for 8–10 days. Ovarian response was monitored using transvaginal ultrasound scan and serum estradiol assay. Human chorionic gonadotropin (hCG) (Chorimon, IBSA, Switzerland) 10,000 IU was administered when at least three follicles reached 18–20 mm in diameter. Ovum pick-up was performed 34–36 h following hCG injection. ICSI was carried out and embryo transfer was performed 2–3 days following ovum pick up.

For the purpose of the study, only clear follicular fluid without significant contamination with blood or culture media was examined after removal of oocytes. Follicular fluid was then centrifuged at 700 g for 10 min at room temperature to remove cellular components. Supernatants were collected and kept at ~80 °C for no more than 1 week until analysis.

The study was ethically approved by the research committee of the faculty of Science, Cairo University.

2.1. Methods

2.1.1. Determination of GSSG and GSH in follicular fluid samples

Five milliliter of the follicular fluid was mixed with 25 ml of the mobile phase which consisted of potassium phosphate buffer acetonitrile at pH 2.7. The mixture was analyzed on Agilent HP 1100 series HPLC (USA) at 210 nm wavelength with flow rate 2 ml/min according to Jayatilleke and Shaw (16). The resultant chromatogram identified oxidized and reduced glutathione positions and concentrations by comparing the samples to the chromatogram of the standard solutions of GSSG and GSH. Glutathione (oxidized and reduced) reference standard (Sigma Chemical Co., Australia) was dissolved in 75% methanol (1 mg/ml) and diluted before use.

2.1.2. Determination of MDA in the follicular fluid samples

Follicular fluid samples were analyzed for the MDA concentration by dissolving 5 ml of the sample in 25 ml mobile phase consisting of 30 mmol potassium phosphate monobasic (K$_2$PO$_4$) and methanol (65–35% phosphoric acid at pH 4) and analyzed using Agilent HP 1100 series HPLC (USA) at 250 nm wavelength with flow rate 1.5 ml/min according to
Karatas et al., (17). The resultant chromatogram identified MDA position and concentration by comparing the samples to the chromatogram of the standard solutions of MDA. MDA standard solution was prepared by dissolving 25 µL tetraethoxypropane (TEP) in 100 ml water to give 1 ml solution. Working standard was prepared by hydrolysis of 1 ml TEP stock solution in 50 ml 1% sulfuric acid and incubation for 2 h at room temperature. The resulting MDA standard of 20 nmol/ml was further diluted with 1% sulfuric acid to yield the final concentration of 1.25 nmol/ml to get the standard for the estimation of total MDA.

2.1.3. Determination of nitrates and nitrites in the follicular fluid samples

Nitrites and nitrates concentrations in follicular fluid samples were determined according to Papadoyannis et al., (18). Five milliliter of the collected follicular fluid was mixed with 25 ml of 0.1 ml NaCl–methanol at volume ratio 45:55, respectively as a mobile phase prepared sample and analyzed on Agilent HP 1100 series HPLC (USA) at 230 nm wavelength with flow rate 2 ml/min. The resultant chromatogram identified each of nitrite and nitrate positions and concentrations by comparing the samples to the chromatogram of the standard solutions. Sodium nitrite and nitrate were used for the preparation of the reference standard with stock concentration 1 mg/ml. Nitrites and nitrates were equal in the mixture solution.

3. Statistical method

Results were expressed as mean ± SD, median and range for quantitative variables and percentages for categorical variables. Categorical variables between the two groups were compared using the chi-square test and continuous variables were compared by using t-test. Bivariate analysis and multiple linear regression analysis were used to examine the association between BMI and ROS and IVF/ICSI outcomes with the presence of confounders. P value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

4. Results

Seventy-four women were enrolled in the study. Apart from BMI, there were no statistically significant differences regarding the basic demographic characteristics between both groups (Table 1).

Clinical pregnancy rate was significantly higher in group I (87%) compared to group II (43%) (P < 0.01, OR: 13; 95% CI 3.54–52). Otherwise, no statistically significant differences between both groups were detected regarding other ICSI parameters and outcome, including total dose of Gonadotropin, serum E2 level on day of HCG, number of retrieved oocytes, MII oocytes and cleaved embryos (Table 2).

As regards, free radicals concentration, the median MDA concentration in the follicular fluid in group II was 676 µmol/l which was three times as high as in group I (221), P < 0.006. NO2/NO3 was significantly higher in group II (1.63 ± 0.2) than in group I (1.47 ± 0.2), P < 0.004. On the other hand, in terms of antioxidant measures, there was no significant difference between the two groups in terms of GSH (406.1 ± 340.7 vs. 501.8 ± 269.2) and GSH/GSSG ratio (0.84 ± 0.59 vs. 0.77 ± 0.57) (P > 0.05) (Table 3).

Bivariate analysis showed a significant positive correlation between BMI and MDA (r = 0.335, P = < 0.01) and an insignificant positive correlation with NO2/NO3 (r = 0.217, P = 0.06), GSH (r = 0.066, P = 0.57). Linear regression analysis showed that each unit of increase in BMI resulted in a 21.9 increase in the level of follicular fluid MDA (r² = 0.083, P = 0.013). In contrast, an insignificant negative correlation was observed between BMI and GSH/GSSG (r = – 0.068, P = 0.5). On the other hand, positive correlation was found between MDA and NO2/NO3 ratio (r = 0.26, P = 0.02). Regarding cycle outcome, using bivariate analysis, there was no correlation between follicular fluid MDA level and total number of oocytes, number of MII oocytes and number of cleaved embryos. However, using binary logistic regression, MDA was found to be a good predictor of the occurrence of pregnancy (P = < 0.01).

5. Discussion

The current study demonstrates that female obesity is associated with enhanced oxidative stress response in the follicular fluid. Obese women had significantly higher MDA and NO2/NO3 ratio compared to non-obese. In addition, BMI showed a strong positive correlation to MDA levels as well as a non-significant positive correlation to NO2/NO3 ratio. NO2/NO3 ratio positively correlated to MDA. Furthermore, BMI could predict 8% of the variations in MDA level (r² = 0.083, if multiplied it by 100 = 8%), each additional unit of increase in BMI was associated with 21.9 increase in follicular fluid MDA. Although weak, the positive correlation between BMI and GSH and negative correlation with GSH/GSSG ratio support the existence of oxidative stress environment with higher BMI.

Our results agree with other studies where female obesity was associated with intensified oxidative stress response in the serum and follicular fluid (19,20). In a study by Borowiecka et al., 2012, mean follicular fluid thiobarbituric acid-reactive substances (TBARS) were significantly higher in obese females who did not get pregnant on IVF (21). Obesity has also been associated with high follicular fluid C-reactive protein, an indicator of inflammation, which is often accompanied with an oxidative stress response (22). In addition, high fat diet was associated with a rise in ROS levels in the cumulus-oocyte complex as well as glutathione depletion in pre-ovulatory oocytes and zygotes of female mice with reduction in the rate of blastocyst formation (3).

The present study revealed a negative impact of female obesity on clinical pregnancy rate in ICSI patients. In addition, with binary logistic regression MDA was found to be a good predictor for the occurrence of pregnancy in obese females. On the other hand, total gonadotropin dose, number of retrieved oocytes, oocyte maturity and embryo cleavage did not differ significantly between both groups. No correlation was found between follicular fluid MDA and any of the latter variables. It has been postulated that the presence of imbalance between oxidant and antioxidant mechanisms in the female reproductive tract negatively affects the endometrium and consequently reduces implantation and pregnancy rates (23). Bediawy et al. 2012, reported significantly higher
pregnancy rate in women undergoing ICSI with lower follicular fluid ROS and higher TAC levels, however, contradictory to our results, follicular fluid ROS significantly correlated to the number of retrieved oocytes (24). In contrast, Attaran et al. 2000, reported higher ROS levels in the follicular fluid of women who became pregnant after IVF, however they stated that lack of a reference range for ROS makes it difficult to discriminate what is considered physiological levels from pathological levels and consequently interpreting its effect on IVF outcome, they concluded that low concentrations of follicular fluid ROS may predict IVF success (15). Lack of effect on oocytes reported in the current study may be explained by the ability of the oocytes to repair DNA damage to some extent that makes it difficult to detect the effect of OS on the oocytes, thus measurement of follicular fluid ROS may be a way to detect this damage (9). It has also been reported that oocyte defense against OS depends on the stage of oocyte development, thus certain stages of oocyte maturity might need some degree of OS to be present in the surrounding environment (25,26).

Finally, some limitations exist in the current study. The small sample size of patients might have affected to some extent ICSI outcome. Also we used pooled follicular fluid rather than individual follicular assessment which might have been a more precise reflection of oocyte related parameters. All our cases had male factor as an indication for ICSI which might have contributed to our pregnancy outcome. However, selecting normal females gave strength to our results by eliminating the influence of other female factors as PCOS and endometriosis that could represent contributing factors to oxidative stress and pregnancy rate. This may also suggest that women who are apparently normal and undergoing ICSI for a male factor may still have indirect factors as obesity that might hinder cycle success.

6. Conclusion

Female obesity aggravates follicular fluid OS which in turn negatively affects the success of ART. Further studies are needed to assess the relationship between female obesity and ART outcome.

References


