Ir J Med Sci (2017) 186:641–646
DOI 10.1007/s11845-016-1523-6

ORIGINAL ARTICLE

Obesity is not a descriptive factor for oxidative stress and viscosity in follicular fluid of in vitro fertilization patients

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Received: 23 January 2016 / Accepted: 25 October 2016 / Published online: 5 November 2016
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Abstract
Background Obesity's impact on micro-environmental oxidative stress and follicular fluid (FF) viscosity and whether or not it has any effect on in vitro fertilization (IVF) success is a matter of debate.
Aims In this study, our aim was to evaluate the levels of oxidative stress markers and the FF viscosity in obese and non-obese patients.
Methods Eighty norm-responder patients undergoing IVF were prospectively grouped according to their body mass indexes (BMI). Group 1 ($n = 40$) and group 2 ($n = 40$) had BMI values of $\leq$24.9 and $\geq$25.0, respectively. Total sulfhydryl (RSH) levels (nmol/m) and the formation of thiobarbituric acid-reactive substances (malondialdehyde, or MDA) (μmol/ml) in FFs were quantified. For the first time in our study, FF viscosity with changing BMI values was also determined.
Results The mean levels of MDA (μmol/ml) and RSH (nmol/ml) were not significantly different between groups (1.37 ± 0.51; 1.51 ± 0.51; $p > 0.05$ for MDA and 0.42 ± 0.30; 0.41 ± 0.20; $p > 0.05$ for RSH, respectively). Similarly, the FF viscosity (centipoise) was not different between groups (1.28 ± 0.28; 1.30 ± 0.19; $p < 0.05$, respectively). Independent of BMI, no correlation was found between FF levels of oxidative markers and the number of oocytes retrieved or the fertilization rates.
Conclusions In our study, we found no difference in the levels of follicular oxidative and anti-oxidative markers or the follicular fluid viscosity with changing BMI values. We also demonstrated that the levels of oxidative stress markers and the viscosity of follicular fluid did not affect clinical outcomes.

Keywords Follicular fluid · Oxidative stress · Body mass index · Malondialdehyde (MDA) · Sulfhydryl group (RSH) · Viscosity

Introduction

Obesity is one of the biggest health problems worldwide. The best known undesirable effects of obesity on reproductive functions include menstrual irregularities, decreased uterine receptivity [1], decreased fecundity [2] and increased pregnancy complications [3, 4], as well as its detrimental effects on embryo and oocyte quality [5–8].

Obesity is related to the accumulation of lipids in non-adipose tissue, leading to systemic oxidative stress and inflammation [9]. In this situation, reactive oxygen species (ROS) cause the oxidation of macromolecular structures such as nucleic acids, lipids and proteins [10]. One of these products is malondialdehyde (MDA), forming as a result of lipid peroxidation. To maintain the tissue's hemostasis as well as to balance ROS's undesirable effects, antioxidant mechanisms take place. Total sulfhydryl groups (RSH)

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constitute an important part of this antioxidant defense. Weight loss was shown to decrease this systemic free radical generation and to increase the antioxidant vitamins in blood plasma [11]. Although the effect of increased body mass index (BMI) on systemic generation of ROS is well defined, only a limited number of studies investigated obesity's effects on oxidative stress markers in the follicular fluid (FF), which constitutes the micro-environment of the oocyte [12].

The effect of FF viscosity is another intriguing subject in female reproductivity. Obesity and oxidative stress can lead to erythrocyte membrane deformabilities and, therefore, increase the blood viscosity [13, 14]. However, to our knowledge, the effect of obesity on FF viscosity has not been investigated before. The data about the relation between the levels of FF viscosity and IVF outcomes are also limited [15, 16].

Therefore, in this study, we aimed to evaluate BMI's effect on oxidative stress markers in FF as well as FF's viscosity and the relation between these markers and FF viscosity to IVF outcomes.

Materials and methods

Participants

Eighty norm-responder patients who were referred for IVF were allocated for this study. The exclusion criteria were any known endocrinopathy, hyperandrogenism, polycystic ovary syndrome, or any systemic disease. The Local Ethics Committee and Institutional Board approved the study. Informed consent was obtained from the patients.

The patients were divided into two groups according to their body mass indexes (BMI). Group 1 ($n = 40$) was defined as patients with BMI $\leq$24.9 and group 2 ($n = 40$) had BMI >25.

Long protocol with gonadotropin-releasing hormone (GnRH) agonist was applied to 58 patients, whereas short protocol with GnRH agonist was applied to eight patients, and GnRH antagonist protocol was applied to 14 patients. Oocyte maturation was triggered using subcutaneous 250 μg of human chorionic gonadotrophin (hCG; Ovitrelle®, Merck-Serono).

Oocyte retrieval was performed 36 h later by transvaginal ultrasonography-guided follicular aspiration. Normal fertilization was identified by the two pronuclei (2PN) 16–18 h following intra-cytoplasmic sperm injection (ICSI). The embryos were transferred on days 3 through 5, depending on clinical situation. All cycles had luteal-phase support with intravaginal progesterone gel (Crinone 8%®, 90 mg vaginal gel, Merck-Serono) beginning on the day of oocyte retrieval. The fertilization rate was defined as the ratio of two pronuclei (2PN) number to the number of metaphase II (MII) oocytes. A positive blood β-human chorionic gonadotrophin (β-HCG) test on day 12 of embryo transfer was defined as biochemical pregnancy, whereas the presence of fetal cardiac activity was defined as clinical pregnancy.

Determination of follicular lipid peroxidation (malondialdehyde-MDA)

For the biochemical measurements and assessment of viscosity, the pooled FF of each patient was used.

The lipid peroxidation level was quantified with the thiobarbituric acid (TBA) reaction method [17]. After centrifugation of aliquots (0.5 ml), the supernatants were mixed with 1 ml of a solution which contained 0.375% (wt/vol) TBA, 15% (wt/vol) tricarboxylic acid and 0.25 N HCL. Following the centrifugation, the supernatants were added to 0.02% (wt/vol) butylated hydroxytoluene to prevent further peroxidation of lipids during subsequent steps. The samples were then heated for 15 min at 100 °C in a water bath. The absorbance of each sample was determined spectrophotometrically at 532 nm. The calculated MDA levels were expressed as μmol/ml.

Determination of follicular total sulphydryl group (RSH) level

The RSH levels were determined using the method of Kurtel et al. [17]. A total of 0.5 ml of each sample was added with 1 ml of a solution which contained 1% sodium dodecyl sulfate, 100 mM Tris–HCl (pH 8.2) and 2 mM ethylenediaminetetraacetic acid (EDTA). Following the incubation and centrifugation, 0.3 mM of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was added and incubated at 37 °C for 15 min. The absorbance of each sample was determined spectrophotometrically at 412 nm. The calculated RSH levels were expressed as nmol/ml.

Viscosity assessment

Viscoelastic properties were determined via the measurement of oscillatory flow in a cylindrical tube called the Vilastic Bioprofiler (Vilastic Scientific, Inc., Austin, Texas, United States). A total of 0.5 ml of FF sample was used.

Before the measurement, the tube was filled with de-ionised water at 37 °C. When the temperature equilibrium was established, the de-ionised water's viscosity was measured and subtracted from the FF sample's viscosity. This was done to eliminate the transport medium's effect on the measurement of the sample's viscosity. All of the measurements were performed under a constant temperature. To establish this, the tube was assured to run under a constant temperature for at least 40 min before all of the

measurements were made. To eliminate the effect of the time interval between gathering the sample and making the measurements, all of the samples were evaluated after a certain and constant time period. The viscosity results were given as centipoise (cP) units.

The primary outcome was the effect of BMI on MDA, RSH levels and FF viscosity. The secondary outcomes were these measurements' correlation to the number of MII oocytes and the fertilization rates.

Statistical analysis

The number of participants needed was determined by referring to the published data of a study, which were similar to our concept. With an estimated deviation of ±10%, we chose a significance level (alpha) value of 0.05, and a power of 0.90 [18]. Based on these results, a total of 60 patients at least were necessary as a sample size.

For statistical analyses, SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL) was used. The one-sample Kolmogorov–Smirnov test was used to analyze the normality for the continuous variables. The comparison between percentages was made using the Chi-squared test. For comparison of the continuous variables, the Mann–Whitney *U* test was used. Data were described as mean ± standard deviation and percentage according to the type of the variable. Pearson correlation analysis was performed to analyze the correlation between follicular markers (RSH, MDA, viscosity) and fertilization rate. A p value <0.05 was accepted as statistically significant.

Results

Patient characteristics

According to the BMI value, the patients' demographic and clinical features are shown in Table 1. No significant differences were found in terms of age or basal FSH, LH and E2 values between groups ($p > 0.05$).

The total gonadotropin dosage, peak estradiol level on the day of hCG administration, endometrial thickness on the day of oocyte retrieval, number of retrieved oocytes, number of MII oocytes, fertilization rates and chemical–clinical pregnancy rates were also comparable between groups (Table 1).

Primary and secondary outcomes

When we assessed the FF levels of RSH and MDA, no statistically significant difference between groups was observed (Table 2). However, when we did a subgroup analysis, the patients that had a BMI value below 18.5 ($n = 6$) had a significantly lower FF viscosity than the other patients in group 1 ($p = 0.001$).

Independent of BMI, we did not observe any correlation between oxidative stress markers and the number of MII oocytes or the fertilization rate. Similarly, no correlation was found between FF viscosity and the number of MII oocytes or the fertilization rate. We also did not observe any correlation between oxidative stress markers and FF viscosity (Table 3).

Table 1 Demographic and clinical features of groups

	Group 1 ($n = 40$)	Group 2 ($n = 40$)	p
Age (years)	29.28 ± 5.55	31.45 ± 5.22	0.041¥
BMI (kg/m^2)	22.09 ± 2.07	28.82 ± 3.57	0.001¥
FSH (IU/ml)	6.54 ± 2.23	5.77 ± 2.42	0.219¥
LH (IU/ml)	4.46 ± 2.36	4.10 ± 1.85	0.200¥
Basal E2 (pg/ml)	59.96 ± 29.58	54.42 ± 20.21	0.575¥
Total gonadotropin dosage (IU)	2223 ± 1527	2394 ± 1573	0.277¥
Peak E2 level (pg/ml)	2003 ± 1162	1958 ± 1316	0.779¥
Endometrial thickness (mm)	10.07 ± 1.90	10.42 ± 2.01	0.411¥
Number of retrieved oocytes (n)	10.20 ± 7.56	10.50 ± 6.36	0.820¥
Number of MII oocytes (n)	8.58 ± 6.30	8.31 ± 5.91	0.748¥
Number of 2PN (n)	4.26 ± 2.86	4.95 ± 4.14	0.731¥
Fertilization rate (%)	53.92 ± 27.47	54.15 ± 25.49	0.950¥
Chemical pregnancy rate (%)	15/40 (37.5%)	16/40 (40%)	0.818#
Clinical pregnancy rate (%)	13/40 (32.5%)	11/40 (27.5%)	0.626#

BMI body mass index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *M2* metaphase 2, *2 PN* two pronuclei, p value <0.05 is statistically significant

¥ Mann–Whitney *U* test was used for statistical analyses. # Chi-squared test was used for statistical analyses

Table 2 The comparison of follicular fluid RSH, MDA levels and the follicular fluid viscosity

	Group 1	Group 2	*p*
RSH (nmol/ml)	0.42 ± 0.30	0.41 ± 0.20	0.892
MDA (μmol/ml)	1.37 ± 0.51	1.51 ± 0.51	0.06
Viscosity (cP)	1.28 ± 0.28	1.30 ± 0.19	0.296

RSH total sulfhydryl groups, *MDA* malondialdehyde, *cP* centipoise, *p* value <0.05 is statistically significant

Discussion

In this study, our aim was to determine BMI's effect on the FF viscosity and oxidant–antioxidant markers in FF of IVF patients as well as their effects on IVF outcomes. In this way, we wanted to determine if obesity affects the oocyte's micro-environment—in other words, 'the follicular fluid'.

The relationship between obesity and infertility is well known along with the association between obesity and systemic oxidative stress. Increasing BMI and oxidative disequilibrium increases the likelihood of female fertility disorders [19, 20]. The studies suggest that increasing BMI values may cause an impairment in the oocyte quality as well as the number of MII oocytes and the rate of fertilization [21–23].

Various explanations have also been made to explain BMI's effects on infertility. Reduced implantation rate [7], obesity-induced changes in reproductive hormones, glucose metabolism, free fatty acids and adipokines [8] are some mechanisms that authors have stressed. However, increased BMI's effect on systemic oxidative stress and its reflection in FF remains unclear.

FF constitutes the micro-environment of the oocyte. Physiologically, ROS are produced in FF during oocyte development and oocyte maturation [24]. Although a physiologic amount is necessary [25], the oxidative disequilibrium may impair the oocyte quality and the fertilization [26, 27].

The data from studies investigating the oxidative stress in FF are controversial. Some suggested that oocyte maturity is not effected by changing levels of ROS [28, 29]. However, some investigators observed a positive correlation between these parameters and concluded that the women who achieved a pregnancy through IVF had significantly higher FF ROS levels than did non-pregnant women [25, 30]. On the other hand, some authors suggested that oocyte maturation showed a positive correlation with low levels of oxidative stress [31].

To our knowledge, besides these studies, the data comparing oxidative stress markers in FF with changing BMI values are limited. In one study, Bausenwein et al. showed that obese women had significantly greater amounts of oxidated low-density lipoprotein (LDL) and significantly higher antioxidant catalase activity in the FF as compared with non-obese women. However, they did not find any difference in the activity of another antioxidant enzyme, SOD [12].

In our study, similar to Bausenwein et al. we did not find any significant difference in terms of RSH and MDA levels in FF based on BMI, which meant that obesity did not cause an oxidative disequilibrium in the follicular micro-environment. However, apart from that study, we also evaluated oxidative stress markers' and FF viscosity's effect on IVF outcomes. As a result, we found no correlation either between oxidative stress markers and the number of mature oocytes or between FF viscosity and the number of mature oocytes.

FF viscosity's effect is another intriguing subject in female reproductivity. In the only related study we found in literature, Fisch et al. found no significant difference in terms of FF viscosity in thawed frozen fluids and in fresh samples and also no correlation between FF viscosity and the presence of oocytes, their maturation grade or their fertilizing capacity [16]. Nonetheless, this study was done with the ignorance of patients' BMI.

Table 3 Correlation analysis of follicular fluid RSH, MDA levels and follicular fluid viscosity with clinical outcomes

	Pearson correlation coefficient	*p*
RSH—number of MII oocytes	−0.055	0.633
MDA—number of MII oocytes	+0.079	0.496
FF viscosity—number of MII oocytes	+0.219	0.056
RSH—fertilization rate	+0.147	0.209
MDA—fertilization rate	+0.086	0.461
FF viscosity—fertilization rate	+0.170	0.146
RSH—FF viscosity	−0.060	0.595
MDA—FF viscosity	+0.125	0.269

RSH total sulfhydryl groups, *MDA* malondialdehyde, *FF* follicular fluid, *p* value <0.05 is statistically significant

As we know, obesity and oxidative stress are associated with increased blood viscosity [32]. Generally, an obesity-related increase in blood viscosity is attributed to the prooxidants and adipocytokines that alter erythrocyte morphology and decrease erythrocyte deformability [33].

This well-defined association between obesity and blood viscosity raised the question of whether or not obesity also affects FF viscosity. Thus, in our study, we compared FF viscosity with different BMI values. However, except for the lean group (BMI <18.5) in the subgroup analysis, we could not find any significant difference in FF viscosity between groups. We believe BMI's lack of influence on FF viscosity, unlike its strong influence on blood, maybe related to the absence of formed elements in FF.

Our study has some limitations. First, the sample size was relatively small in the lean group (BMI <18.5). Therefore, the low viscosity in this subgroup's FF cannot be generalised to the general population. Second, the oxidative stress and FF viscosity was analyzed in the pooled aspirated FF for each patient, so it is possible that it may not reflect each individual follicle. From this point of view, to assess the oxidative stress markers in the FF of every follicle and its effect on every oocyte and IVF outcome might be more valuable. Third, we did not include patients who had any known endocrinopathy. However, obesity has a close relation with diabetes mellitus which also has obvious adverse effects on oxidative stress and fertility. Therefore, these patients could have been included as a separate group with some metabolic parameters such as homeostatic model assessment (HOMA) or C-reactive protein (CRP) and their relation with IVF outcomes could have been evaluated as well.

This study's strength is that it includes patients from different BMI values and investigates the effect of BMI on oxidative stress markers in FF and, for the first time in the literature, follicular fluid viscosity. Moreover, to our knowledge, for the first time in literature, this study also investigates the effect of FF levels of oxidative stress markers and FF viscosity on IVF outcomes. Thus, we believe that this study will provide a useful contribution to its area of research.

Conclusions

In conclusion, we have shown that obesity is not a descriptive factor for oxidative stress or the viscosity in follicular fluid in IVF patients. Moreover, none of these parameters have any effect on IVF outcomes. Yet, further studies are needed to confirm our findings.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest All authors declare that they have no conflict of interest.

Informed consent An informed consent was obtained from all participants prior to the study.

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