EFFECT OF BMI OF INFERTILE FEMALES ON ROS LEVEL IN EMBRYO CULTURE MEDIA

Hanan Abdulrazzaq Abdulazeez¹, Dr. Muayad Sraibet Abbod², Dr. Mufeda Ali Jwad³,
¹,²,³High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq

ABSTRACT

Background: Infertile couples often turn to assisted reproductive techniques (ART) to improve their chances of conception. Elevated reactive oxygen species (ROS) levels correlate with the poor fertility outcomes seen in the assisted reproductive technology setting. Because oxidative stress (OS) from excessive ROS generation has been implicated in the pathogenesis of obesity, so a better understanding of ROS-mediated reactions and their impact on embryonic development is important to ensure optimal outcomes.

The objectives of the study: The main objective was to demonstrate the potential occurrence of an association between BMI and ROS levels and its effect on embryo quality.

Patients and Methods: Eighty women, under the age of 45 underwent controlled ovarian hyperstimulation using antagonist protocol and ICSI-ET; in High institute for Infertility Diagnosis and Assisted Reproductive Technologies. They were classified into three groups according to the weight of the women involved. The oocytes were retrieved using an ultrasound guide and were fertilised via injection of sperm inside the follicle (ICSI). ROS serum level was analysed on the day of OPU and ROS levels in a sample of each embryo culture dish measure on the day of embryo transfer using enzyme-linked immunosorbent assay. Clinical IVF cycle outcomes (embryo grading and pregnancy rate) were compared between the three BMI groups.

Results: There were significant differences among the three groups in culture media ROS levels ($p=0.017$) with higher levels in obese patients, however, there were no significant differences among the three groups regarding the grade of embryos. Although the pregnancy rate was highest (43.5%) in the normal weight group and lowest (18.2%) in the obese group, there was no significant difference among these groups.

Conclusion: Females with higher BMI had higher ROS (Reactive oxygen species) levels and this may account for their poorer treatment outcomes in IVF cycles when compared with normal BMI individuals.

Keywords: Reactive oxygen species (ROS), Body mass index (BMI), Assisted reproductive techniques (ART).

I. INTRODUCTION

Infertility is defined by the WHO as failure to achieve a clinical pregnancy (CP) after 12 months or more of regular unprotected sexual intercourse. The prevalence of infertility ranges from 12% to 24%, depending on the study (Merviel et al., 2020).

Obesity is a major contributor to a variety of underlying etiologies associated with infertility and has a significant negative effect on Assisted Reproductive Technology (ART) outcomes (Shehata, 2017).

As reproductive medicine continues to advance, with continual improvement in in vitro fertilization (IVF) success rates, some fundamental physiological questions, such as whether body mass index (BMI) impacts fertility and pregnancy outcomes, remain unanswered, therefore, understanding this potential impact of BMI remains critically important (Kudesia et al., 2018).
In some countries, over 50% of reproductive-aged women are either overweight (body mass index (BMI) 25–29.9 kg/m²) or obese (BMI ≥ 30 kg/m²). Compared to normal-weight women (BMI 18.5–24.9 kg/m²), obese women have a threefold risk for infertility (Ben-Haroush et al., 2018).

In women, obesity has been linked to an increased risk of menstrual dysfunction, anovulation, and infertility, (Legge et al., 2014) so affects female reproductive function in many ways. For example, adiposity can increase the peripheral aromatization of androgens to estrogens and decrease the hepatic synthesis of sex hormone-binding globulin (SHBG) protein, leading to excessive secretion of luteinizing hormone (LH), an increased ratio of androgens to estrogens, and an altered endocrine environment leading to impaired folliculogenesis (Zhang et al., 2017).

However, there are conflicting reports on the effect of obesity on oocyte quality, embryo development, lower number of mature oocytes, lower implantation and pregnancy rates (Sathya et al., 2010).

These inconsistencies may be due to genetic, environmental, and ethnic differences in the study participants. (Zhang et al., 2017).

The physiological process of oxygen consumption inevitably generates ROS in cellular respiration for energy production. Although ROS, in limited amounts, are considered to mediate inter-and intracellular signalling, it has been reported that elevated ROS levels and the resulting oxidative stress are associated with poor or arrested embryo development. Furthermore, oxidative stress has recently been determined to be one of the major factors detrimental to ART outcomes (Shih et al., 2014).

Oxidative stress in the field of productivity has been extensively considered in association with sperm properties and male subfertility; regardless, the appraisal of oxidative stress in association with oocyte characteristics and women subfertility is far not as much as that stressed over male subfertility (Hindal et al., 2018).

So the need to identify the most viable embryo following in vitro fertilization (IVF) is crucial to enhance successful pregnancy rates. Identification and transfer of single embryos of known viability and high developmental potential can avoid multiple pregnancies. The standard method of identifying the best embryos has been morphology (Lan et al., 2019).

Infertility caused by oxidative stress has been widely studied for years, and multiple studies analyzing its clinical effect have been performed, concerning both natural fertility and assisted reproductive techniques. (Ribas-Maynou and Yeste, 2020).

II. MATERIALS AND METHODS

The present prospective study has involved a total of 80 women aged between (20–45 y) who underwent controlled ovarian hyperstimulation during a period of study which started on November 2020 up to April 2021, and was done at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University. The study was approved by the Local Medical Ethical Committee. The demographic and baseline clinical information will be collected included the following: age of female patient intending pregnancy, type of infertility, anti-Müllerian hormone (AMH) levels, basal follicle-stimulating hormone (FSH) levels, basal luteinizing hormone (LH) levels, basal estradiol (E₂) levels, basal antral follicle count (BAFC), sperm volume, total motile sperm, sperm morphology, peak serum E₂ levels, number of oocytes retrieved, use of ICSI, stage of an embryo at transfer (cleavage stage vs. blastocyst), number of embryos transferred, and pregnancy outcome.

All participants in the current study were divided according to BMI into three groups

and passed through the routine ICSI procedure including clinical evaluation (history, examination and investigation), controlled ovarian stimulation, triggering of ovulation, oocyte retrieval under general anesthesia, oocyte denudation, oocyte maturation evaluation, intracytoplasmic sperm injection of mature (MII) oocytes, evaluation of fertilization and cleavage and embryo grading, embryo selection and embryo transfer, luteal phase support, beta hCG determination (to document biochemical pregnancy). Sperm processing has been carried after oocyte denudation. Oocyte and embryo quality were assessed depending on microscopical morphological criteria. Measurement of ROS serum level on the day of OPU and ROS levels in a sample of each embryo culture dish on the day of embryo transfer was done. After ICSI, embryo development, including the four-cell stage (45–
46 hours), eight-cell stage (69–70 hours), and blastocyst stage (118–120 hours), were observed. The embryos at the cleavage stage were classified. On Day 2 and Day 3, embryos consider good when they exhibit fragmentation of <20% and equal-sized blastomeres.

The clinical pregnancy rate will be calculated by dividing the number of cycles with fetal cardiac activity on ultrasound by the number of embryo transfer cycles, and the implantation rate will be calculated by dividing the number of gestational sacs on ultrasound by the number of embryos transferred (Lan et al., 2019).

### III. RESULTS

A total of 80 patients were included in the analysis. The BMI of the patients ranged from 20.2 - 36.7 kg/m²; patients mean BMI were 26.82 ± 3.29 kg/m².

All patients in the current study were divided according to BMI into:

1. Normal weight patients (23 patients with BMI 18 - 24.9).
2. Overweight patients (46 patients with BMI 25 - 29.9)
3. Obese patients (11 patients with BMI > 30)

Regarding the demographic parameters there was a significant difference in mean body mass index (BMI) (p=0.023) and body mass index classes (p=0.047) between pregnant and non-pregnant females with lower BMI in pregnant patients, meanwhile (age, duration of infertility, type, and cause of infertility) the statistical analysis showed no significant differences between pregnant and non-pregnant ICSI patient groups with (p=0.065), (p=0.790), (p=0.348) and (p=0.312) respectively, as shown in Table 1 and Figure 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pregnant women n=24</th>
<th>Non-pregnant women n=56</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (Mean±SD)</td>
<td>30.67 ± 5.35</td>
<td>33.38 ± 6.15</td>
<td>0.065</td>
</tr>
<tr>
<td>BMI (Kg/m²) (Mean±SD)</td>
<td>25.56 ± 3.25</td>
<td>27.36 ± 3.10</td>
<td>0.023*</td>
</tr>
<tr>
<td>Normal weight</td>
<td>14 (58%)</td>
<td>13 (23.2%)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>10 (34%)</td>
<td>34 (60.7%)</td>
<td>0.047*</td>
</tr>
<tr>
<td>Obese</td>
<td>2 (8%)</td>
<td>9 (16%)</td>
<td></td>
</tr>
<tr>
<td>Duration of infertility (years) (Mean±SD)</td>
<td>3.15 ± 1.23</td>
<td>3.93 ± 1.44</td>
<td>0.790</td>
</tr>
<tr>
<td>Type of infertility n. (%)</td>
<td>Primary 18 (75 %)</td>
<td>Primary 47 (83.9 %)</td>
<td>0.348</td>
</tr>
<tr>
<td>Cause of infertility n. (%)</td>
<td>Female causes 14 (58.3 %)</td>
<td>Female causes 30 (53.6 %)</td>
<td></td>
</tr>
</tbody>
</table>
Comparison of demographic features between pregnant and non pregnant women

Figure 1: Comparison of demographic features between pregnant and non pregnant women

Comparison of embryo characteristics between normal weight, overweight and obese patients

The comparison of patients’ embryo characteristics between normal weight, overweight and obese patients was demonstrated in Table 2, the results showed no significant differences between the three groups in embryo transfer day \( (p=0.244) \), the number of transferred embryos \( (p=0.444) \), grade I embryos \( (p=0.558) \), grade II embryos \( (p=0.166) \) and grade III embryos \( (p=0.471) \).

Table 2: Comparison of embryo characteristics according to BMI

<table>
<thead>
<tr>
<th></th>
<th>Normal weight n=23</th>
<th>Over weight n=46</th>
<th>Obese n=11</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of ET n. (%)</td>
<td>Day 3=17(74%)</td>
<td>Day 3=34(74%)</td>
<td>Day 3=11(100)</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td>Day 5=6 (26%)</td>
<td>Day 5=12(26%)</td>
<td>Day 5=0</td>
<td></td>
</tr>
<tr>
<td>No. of ET (Mean ± SD)</td>
<td>3.13 ± 0.87</td>
<td>2.8 ± 1.05</td>
<td>3.0 ± 1.18</td>
<td>0.444</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI embryo (Mean ± SD)</td>
<td>2.17 ± 0.98</td>
<td>2.0 ± 1.39</td>
<td>2.46 ± 1.37</td>
<td>0.558</td>
</tr>
</tbody>
</table>
Comparison of serum and culture media ROS between normal weight, overweight and obese patients

Serum and culture media ROS in normal weight, overweight and obese patients were illustrated in Table 3 and Figure 2. According to the results, there were significant differences between the three groups in culture media ROS levels ($p=0.017$) with higher levels in obese patients however, there were no significant differences in serum ROS level between the three groups of patients ($p=0.542$) with lower level in obese patients.

Table 3: Comparison of serum and culture media ROS according to BMI

<table>
<thead>
<tr>
<th>ROS level (U/l)</th>
<th>Normal weight n=23</th>
<th>Over weight n=46</th>
<th>Obese n=11</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ROS</td>
<td>690 ± 307</td>
<td>746 ± 623</td>
<td>560 ± 102</td>
<td>0.542</td>
</tr>
<tr>
<td>Culture media ROS</td>
<td>285.2 ± 69.8</td>
<td>271.5 ± 45.1</td>
<td>329.1 ± 83.9</td>
<td>0.017*</td>
</tr>
</tbody>
</table>

ROS: Reactive oxygen species; *: $p$ value < 0.05 (significant)

Correlations between serum and culture media ROS levels and embryo grading

The correlation between serum and culture media ROS and embryo grading was shown in Table 4 and Figure 3, according to the results there were significant and inverse correlation between serum ROS level and number of grade I embryos ($r=-0.230$, $p=0.041$) however there was no significant correlation between serum ROS levels and grade II and grade III embryos ($r=-0.098$, $p=0.406$) and ($r=0.067$, $p=0.626$) respectively. Similarly there was also negative significant correlations between culture media ROS and grade I embryos ($r=0.297$, $p=0.048$) and no significant correlation with grade II and grade III embryos ($r=0.148$, $p=0.208$) and ($r=0.039$, $p=0.778$) respectively.

Table 4: Correlations between serum and culture media ROS levels and embryo grading
ROS level | GI embryos | GII embryos | GIII embryos
--- | --- | --- | ---
Serum ROS levels | $r = -0.230$ | $r = 0.098$ | $r = 0.067$ |

$p = 0.041^*$

$p = 0.406$

$p = 0.626$

ROS level | GI embryos | GII embryos | GIII embryos
--- | --- | --- | ---
Culture media ROS levels | $r = -0.297$ | $r = 0.148$ | $r = 0.039$ |

$p = 0.048^*$

$p = 0.208$

$p = 0.778$

ROS: Reactive oxygen species; $r$: Pearson’s correlation coefficient; G: Grade; *: $p$ value < 0.05 (significant)

Figure 3: Correlations between serum ROS levels and GI embryos

**Comparison of pregnancy rate between normal weight, overweight and obese patients**

The comparison of pregnancy rate between normal weight, overweight and obese patients as illustrated in Table 5 and Figure 4, the results demonstrated higher pregnancy rate in normal weight patients (43.5%) and lower rate in obese patients (18.2%), however, there were no significant differences in pregnancy rate between the three groups of patients ($p=0.217$).

Table 3: Comparison of pregnancy rate according to BMI
Parameter | Normal weight | Overweight | Obese | p-value
--- | --- | --- | --- | ---
Pregnant n.=24 | 10 (43.5%) | 12 (26.1%) | 2 (18.2%) | 0.217
Non-pregnant n.=56 | 13 (56.5%) | 34 (73.9%) | 9 (81.8%) |

Figure 4 : Comparison of pregnancy rate according to BMI

IV. DISCUSSION

Data from 80 participants were analyzed in this prospective cohort study and divided according to BMI into three groups. Determination of ROS levels in both the serum and culture media of infertile women undergoing IVF with ovarian stimulation and direct comparison with the quality grade of the obtained embryos were done.

Regarding the demographic parameters (other than BMI of patients) the statistical analysis showed no significant differences between pregnant and non-pregnant ICSI patient groups because all the three groups were comparable concerning age, duration of infertility, female and male causes of infertility.

Obesity has become a worldwide epidemic. Consequently, an increasing number of overweight and obese women are seeking fertility through assisted reproduction technology. Thus, the impact of raised BMI on the outcome of IVF treatment is of interest to patients, clinicians and policymakers (Rittenberg et al., 2011). Obese infertile women who undergo ART face some difficulties during the treatment. Several studies have shown that the adverse outcomes of ovarian stimulation in obese women are poor quality of oocyte and embryo, decreased intrafollicular human chorionic gonadotrophins concentration, decreased peak estradiol levels, decreased number of mature oocytes, decreased incidence of embryo transfer, and decreased the number of transferred embryos (Ozcan Dag and Dilbaz, 2015).

Embryo characteristics among normal weight, overweight and obese patients were investigated, the results showed no significant differences among the three groups on the day of embryo transfer, the number of transferred embryos, grade I embryos, grade II embryos and grade III embryos.
The preimplantation embryo is affected by an obese environment. Comparison of human IVF cycles with autologous oocytes show that obese women are more likely to create poor quality embryos, elevated leptin levels in obese women may exert a direct negative effect on the developing embryo (Broughton and Moley, 2017). Maternal obesity has been linked to several increased risks to the mother, embryo, and fetus. Igosheva et al (2010) reported a decline in fertility and obscured progression of the developing embryo. Both the quality and quantity of mitochondria are therefore an essential prerequisite for successful fertilization and embryo development (Agarwal et al., 2012).

Early studies documented that BMI had no influence on in vitro fertilization (IVF) outcomes, which was argued by more recent researches that suggested an opposite conclusion. The influence of obesity on the outcomes of IVF/ intracytoplasmic sperm injection (ICSI) remains controversial (Cui et al., 2016).

Several factors are contributing to these discrepancies between studies, including small sample sizes, differences in IVF stimulation protocols, varying BMI classification systems, and inconsistently defined outcome measures. In a recent systematic review, Rittenberg et al. compiled data from 33 studies and demonstrated significantly lower clinical pregnancy and live birth rates among overweight and obese women than among women of normal BMI (Legge et al., 2014).

Metwally et al. demonstrated that there is an association between obesity and poor embryo quality in women below 35 years of age.

Other researchers found that women who are overweight or obese (BMI > 25 kg/m²) had a poorer outcome following IVF treatment compared with women with normal BMI (Rittenberg et al., 2011).

A comparison of serum and culture media ROS among normal weight, overweight and obese patients demonstrated that there were significant differences between the three groups in culture media ROS levels (p=0.017) with higher levels in obese patients, however, there were no significant differences in serum ROS level among the three groups of patients (p=0.542).

Although physiological concentrations of ROS are necessary for normal reproductive function in vivo, in vitro manipulation of gametes and embryos may expose these cells to excess ROS, generated by endogenous or exogenous environmental factors. Endogenously, the gametes and the developing embryo become sources of ROS, which also can be generated by spermatozoa and leucocytes, as well as by events such as sperm-mediated oocyte activation and activation of the embryonic genome (Lan et al., 2019). Also, the overproduction of ROS from accumulated fat leads to increased oxidative stress in the blood of overweight (Vezzoli et al., 2019).

Oxidative stress from excessive ROS generation has been implicated in the pathogenesis of obesity. As major energy producers for cells, the mitochondria synthesize ATP via oxidative phosphorylation. Adverse effects of maternal BMI on mitochondria in the oocyte could negatively influence embryonic metabolism. Maternal obesity has been linked to several increased risks to the mother, embryo, and fetus (Agarwal et al., 2012).

Additionally, in the present study, we noticed there was a significant and inverse correlation between serum ROS level and a number of grade I embryos (r= - 0.230, p= 0.041). Similarly, there was also negative significant correlations between culture media ROS and grade I embryos (r= 0.297, p= 0.048).

Many studies have demonstrated a negative correlation of elevated ROS levels with embryo quality; others have determined a positive correlation, whilst others report that lower levels predict decreased fertilization potential thus reduced oocyte competence, presumably leading to poor embryo quality in the occurrence of fertilization. Recently, Elizur et al. assessed ROS, with the observation that the highest H2O2 values resulted in embryos of poor quality and the lowest ROS values corresponded to empty follicles; the authors linked the presence of increased ROS levels with follicular ageing, incompetence to produce good embryos, and atresia (Siristatidis et al., 2016).

Although morphologic scoring is the best available method of embryo selection, measuring markers of oxidative stress, such as ROS levels, in culture media may be a non-invasive alternative or may be combined with conventional morphology scores to provide more precise embryo selection. Another study, however,
demonstrated that ROS levels in culture media are not significantly associated with zygote score, embryo quality, blastocyst formation or arrest, or chances of conception. (Lan et al., 2019).

Effects of obesity on the endometrium and the embryo may have an additive negative impact on pregnancy outcomes (Broughton and Moley, 2017).

A comparison of pregnancy rate according to BMI groups was done, the results were demonstrated that although there are no significant differences in pregnancy rate among the three groups of patients (p=0.217), there were a higher pregnancy rate in normal weight patients and a lower rate in obese patients.

Previous studies that addressed the correlation between obesity and reproductive outcomes have been controversial. A study showed that the clinical pregnancy rates resulting from the transfer of fresh SET were comparable in normal weight, overweight, and obese women. Some studies reported decreased implantation and pregnancy rates, increased miscarriage rates, and poor pregnancy outcomes in obese women. Those unfavourable outcomes have been attributed to multiple factors including adverse effects of obesity on oocyte and embryo quality, poor ovarian response necessitating increased gonadotropin injections, and lower numbers of collected oocytes, as well as impaired endometrium (Ben-Haroush et al., 2018).

Other studies express no statistical difference was found among these groups in clinical pregnancy rate, Provost et al. found that clinical pregnancy rates in women whose BMI was >30 kg/m2 were lower than women with normal BMI (Zhou et al., 2020).

In another study, the authors did not find any impact of female overweight or obesity on the CP. It is probable that after the age of 36, other factors have a greater influence than BMI on the relative decrease in the likelihood of pregnancy; this would explain results reported by Sneed et al. and Zander-Fox et al.

In contrast, other investigators have failed to show the effect of BMI on pregnancy rates. Indeed, Legge et al., Parent et al., Ozekinci et al. and Ben Haroush et al. found that BMI was not associated with the CP rate (Merviel et al., 2020).

V. CONCLUSION

This study demonstrates that raised BMI has an adverse effect on IVF treatment outcomes. The ROS levels in culture media are associated with embryo development outcomes. Analysis of the ROS levels in culture media may be used as another quality assessment procedure in IVF laboratories.

REFERENCES