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The role of superoxide dismutase on pregnancy rates of women undergoing intrauterine insemination

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ABSTRACT

Background: Recently, assisted reproductive technology has been widely accepted as a well-established procedure to treat infertility. Unfortunately, the success rate is still below expectations, whereas the rate of infertility has risen steadily. Intrauterine insemination procedure is the highest performed compared to other procedures, but the success rate is still lowest, around 10%. One cause of failure has been thought to occur due to lack of oocytes and embryos protection from cleaning of oxygen radicals. Reactive oxygen species negatively affect the maturation and interaction of gamete, fertilization, as well as acceleration of pathological states of the reproductive tract. On female reproduction, an antioxidant is essential for the maturation and quality of oocyte, implantation, placentation, fetal growth, and development of the organ. Concentrations of antioxidants have an important role in the reproduction, and interventions to reduce the influence of reactive oxygen species can improve the quality of embryos and their implantation.

Objective: To determine the effect of antioxidants (superoxide dismutase [SOD]) in ovarian stimulation response and pregnancy rates in women undergoing intrauterine insemination.

Material: Women undergoing intrauterine insemination procedures and who met the criteria of research were randomly and in double-blind divided into two groups, that is, SOD group and placebo group. During ovarian stimulation, 20 patients randomly selected were administered capsules containing 250 mg SOD (glisodin®, Kalbe Pharmaceutical, tbk.) twice a day, and the remaining 20 patients were given the same dose and color capsules containing placebo. Furthermore, ovarian activity and endometrial thickness were monitored by regular transvaginal ultrasound scans. A dose of 5000 U of hCG (Profasi, Serono Pharmaceuticals Ltd., UK) was administered when the leading cohort of follicles reached a diameter of 18–22 mm. Intrauterine insemination with prepared sperms was then performed 34–36 hours following hCG using Friedman catheter. Biochemical pregnancy is determined at day 15 after insemination with the cut-off point 25I U/mL. The data are compared between two groups and analyzed using SPSS 16 with a 95% confidence interval.

Result: Forty women underwent intrauterine insemination cycles, with 20 cycles administered to each group, SOD and placebo. There were five (12.5%) positives for chemical pregnancies, four (80%) in SOD, and one (20%) in placebo. The pregnancy rates tend to be higher in the SOD group than in the placebo group, even though it was not significantly different ($p = 0.151$). The ovarian stimulation determined by the number of growing follicles and endometrial thickness showed that more follicles (≥ 6 follicles) were found in SOD group ($n = 10$, 58.8%) than in placebo ($n = 7$, 41.2%); however, it was not statistically significant ($p = 0.075$). Endometrial thickness of 10 mm or more is statistically significant ($p = 0.017$), which is higher (79.2%) in SOD compared with placebo (20.8%) and yields significantly ($p = 0.0251$) greater pregnancy rates ($n = 4$, 80%) than placebo ($n = 1$, 20%).

Conclusions: SOD provides better stimulation response and increased pregnancy rates in intrauterine insemination program.

Keywords: antioxidant, ovarian stimulation, pregnancy rate

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INTRODUCTION

Assisted reproductive techniques (ARTs) have been widely used and allowed many infertile couples to realize their dream of having a biological offspring. Intrauterine insemination (IUI) is a stage of infertility treatment procedures most widely performed, but the success rate per cycle was still lowest compared to other procedures in ART. These undesirable outcomes encourage researchers to find reasons for failure and a key finding has been that failures occur due to lack of oocytes and embryos protection due to oxygen radicals cleaning. The group of reactive oxygen species (ROS) has been found to be an important factor that negatively affects the outcome and, currently, is allegedly to be one of the causes of female infertility.[1] ROS may affect the maturation and development of the oocyte, follicular atresia, corpus luteum functions, and luteolysis. In female reproduction, an antioxidant is important for the oocytes maturation and quality, implantation, placentation, fetal growth, and organ development. Therefore, the concentration of antioxidant has an important role in reproduction and interventions to reduce the impact of ROS, which in turn can enhance the quality of embryos and implantation. Oxidative stress mediates the acceleration of pathology in the female reproductive tract, similar to its involvement in male infertility. ROS are very influential in the regulation of gamete interaction and the success of fertilization.[2] Oxidative stress affects the quality of oocytes and embryos and, therefore, the rate of fertilization.[1] These results support previous research by Oyawoye,[3] which showed that levels of total antioxidant capacity (TAC) improves fertilization in women undergoing ART.[3]

ROS are derived from various sources, that is, from metabolic activity of embryonic and non-embryonic. ROS in mitochondria are an yield of oxidative phosphorylation. Embryos exposed to high levels of oxygen can lead to transmutation of mitochondrial mRNA.[1] ROS can arise in human endometrium and production changes during the menstrual cycle and pregnancy. ROS stimulate endometrial stromal cells (ESC) to produce prostaglandin F2α (PGF2α), which is involved in the breakdown/disruption of the endometrium (endometrial breakdown).[4] Tatone et al. in a study of 40 women reported that ageing may lead alteration in granulosa cells that is mediated by oxidative stress. In the case of women aged 38 years and above, reducing mRNA that encodes superoxide dismutase (SOD) and catalase is involved in the reduction of its expression at the protein level. Defect in mitochondria of granulosa cells and lipids reduction is due to alighting in defense mechanism against ROS in granulosa cells during the reproductive age.[5]

In female reproduction, an antioxidant is important for the maturation and quality of the oocyte, implantation, placentation, fetal growth, and organ development. Antioxidants also play a role in sexual development, ovulation, and the menstrual cycle. Therefore, ROS disrupt oocyte maturation, ovulation, and play a role in luteolysis and atresia follicle; in addition, the concentration of antioxidant has an important role in reproduction and interventions to reduce the impact of ROS, which in turn enhances the quality of embryos and implantation.[6]

Superoxide dismutase (SOD) is an enzyme that catalyzes dismutation of superoxide into oxygen and hydrogen peroxide. Therefore, SOD is an antioxidant that conserves almost all of the cells exposed to oxygen. As an antioxidant, there are two types of SOD—copper–zinc SOD (CuZnSOD) found in the cytosol and manganese SOD (MnSOD) contained in mitochondria—present in the human endometrium; CuZnSOD controls PGF2α production by preventing the accumulation of ROS in the cytosol and MnSOD acts to protect cells from the clearance of ROS in mitochondria.[7] One study showed that deficiency of SOD in female mice homozygous mutant decreases the proportion and the number of monthly menstruation.

Low levels of selenium glutathione peroxidase (SeGPx) is found in the follicular fluid of women with unexplained infertility. By contrast, SeGPx level is higher in follicles with fertilized oocytes than in follicles with unfertilized oocytes. Enhancement of antioxidants consumption (indicating increased ROS activity) occurs during the incubation of embryos with bad quality.[3] Factor that into the impact of oxidative on native or assisted fertility, then antioxidant strategies presumably suppress both intra- and extra-cellular impacts. Currently, there are no studies that report the effect of antioxidants on the outcome of pregnancy in women who underwent IUI. Therefore, this study was performed to assess whether the antioxidants in women who underwent IUI may improve the pregnancy rate compared with the control group.

MATERIAL AND METHOD

This study was a double-blind controlled clinical trial, which compared the biochemical pregnancy rate between two groups of women: women who underwent IUI procedure and a control group. A total of 40 women who underwent IUI took part in this study. They were divided into two groups with consecutive sampling based on a sequence of randomized blocks. The study was conducted at the Division of Fertility and Reproductive Endocrinology, Department of Obstetrics and Gynecology Sanglah Hospital/Udayana university, Denpasar-Bali. Between July 2008 and October 2010, a total of 40 consecutive patients undergoing IUI were enrolled; informed consents were obtained from the women who met the inclusion criteria. The criteria for inclusion in the study were primary or secondary infertility with normal ovarian reserve, sperm factors, and unexplained infertility.

All the 40 patients were stimulated with the oral administration of 50 mg of clomiphen citrate started on day 3 for a period of 5 days and administrated subcutaneously 75 iu of FSHr (Gonal-F®, Merk, Serono) every 2 days. A total of 20 patients who were randomly selected were administered capsules containing 250 mg SOD (glisodin®, Kalbe Pharmaceutical, tbk.) twice a day during stimulation and were categorized as the treatment group. The remaining 20 patients entered as control group and were given the same dose and color capsules containing placebo. Furthermore, ovarian activity and endometrial thickness were monitored by regular transvaginal ultrasound scans. A dose of 5000 U of hCG (Profasi, Serono Pharmaceuticals Ltd., UK) was administered when the leading cohort of follicles reached a diameter of 18–22 mm. IUI with prepared sperms was then performed 34–36 hours following hCG using Friedman catheter.

Sperm was prepared by washing and swim-up methods. The procedure is simple; semen is washed with a sterile medium added with human albumin. After the fluidification of the sample, the entire volume is divided into fractions of not more than 2 ml into centrifuge tubes. The sterile medium of the equal volume is added in each tube and gently mixed with a sterile pipette. After that, the samples are centrifuged at 300 g for 10 minutes and then the supernatant is very carefully removed with a sterile pipette. The pellet is re-suspended in 1 ml of the medium, gently mixed and centrifuged again for 5 minutes at 300 g. The supernatant is removed again and the final pellet is re-suspended in the sterile medium. The swim-up is preferred if the semen sample has a normal number of good sperms (normozoospermia). The sperms are selected based on their motility and the capability to swim out of the seminal plasma. After the fluidification of the sample, the entire volume (well-mixed) is divided into fractions of 1 ml into centrifuge tubes. A quantity of 1.3 ml of culture medium is placed over the semen. The tubes are then placed in the incubator, inclined at an angle around 45° and incubated at 37°C for 30–60 minutes. After the tubes are returned to their vertical position, 1 ml of the supernatant from each tube is gently removed, aspirating the sperms from the upper meniscus downwards with a sterile pipette. A quantity of 2 ml of medium is added to the supernatant of each tubes and then centrifuged at 300 g for 10 minutes. The supernatant is removed again and the pellet is re-suspended in the sterile medium.

Blood test was performed for all participants on day 15 after the insemination to determine the level of β hCG. Biochemical pregnancy was considered positive when its level was more than 25 mIU/mL. Data were recorded on data collection sheet, then the response of stimulation, chemical pregnancy rate, and other factors that could affect the outcome of IUI were analyzed and compared for both groups. All analyses were performed with SPSS (SPSS Inc., Chicago). Statistical significance was defined as $p < 0.05$.

Table 1 Pregnancy Rate in Treatment Group and Control Group

Group	Biochemical pregnancy		P
	Positive N (%)	Negative N (%)	
SOD	4 (20.0)	16 (40.0)	0.151
Placebo	1 (5.0)	19 (47.5)	
Total	5 (12.5)	35 (87.5)	

Table 2 Growth in the Number of Follicles with Transvaginal Ultrasound Scanning on Day hCG in the Treatment Group and the Control Group

Group	Number of follicles		P
	< 6 Follicles N (%)	≥ 6 Follicles N (%)	
SOD	10 (43.5)	10 (58.8)	0.075
Placebo	13 (56.5)	7 (41.2)	
Total	23 (57.5)	17 (42.5)	

RESULTS

Pregnancy rate

In this study, 26 couples with primary infertility and 14 with secondary infertility were recruited. The sample consisted of 27 women aged below 35 years, and 12 of them were classified as treatment group. The remaining 13 women were aged 35 years or older, and 8 of them were classified as the control group. The pregnancy rate was 20% ($n = 4$) in the treatment group and 5.0% in the control group (Table 1).

Ovarian response

As in natural cycle, the stimulated cycle is expected to yield a synchronous development of ovarian follicles and endometrium to facilitate pregnancy. Therefore, this study also compared the response to the stimulation of follicullar and endometrial growth, further to pregnancy rates in both groups.

Table 2 shows multifollicles (≥ 6 follicles) are more common in the treatment group (58.8%) than in the control group (41.2%). In contrast, ovarian stimulation that yields less than six follicles was found to be more in control group (56.5%) than in treatment group (43.5%). Although statistically not significant ($p = 0.075$), there is a greater likelihood that the administration of SOD on ovarian stimulation increase the number of follicles.

During normal or stimulated cycles, endometrial growth was set ready to receive the embryo in the process of implantation. The thickness of endometrium is one of the clues that can be easily measured to determine its receptivity. Using the findings reported in past literature, we measured and divided the endometrial thickness with a 10-mm cut-off point, on the day of hCG administration as shown in Table 3.

After observing the development of follicles and endometrial thickness in both groups, we observed that there were four pregnancies in the SOD group, with three (75%) of them originating from the group with six or more follicles. Although this was not statistically significant ($p = 0.171$), it was safe to conclude that pregnancies tended to occur more in SOD group which possessed more number (≥6) of follicles. Synchronization of follicular and endometrial development took place during

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Table 3 Distribution of Endometrial Thickness in Both Groups. Endometrial Thickness of 10 mm or More Was Significantly (p = 0.017) More Common in the Treatment Group (79.2%) Compared to the Control Group (20.8%)

Group	Endometrial thickness < 10 mm N (%)	Endometrial thickness ≥ 10 mm N (%)	P
SOD	1 (6.2)	19 (79.2)	
Placebo	15 (93.8)	5 (20.8)	0.017
Total	16 (40.0)	24 (60.0)	

Table 4 Correlation of Follicle and Endometrial Development with Pregnancy Rate in the Treatment Group and the Control Group

	Biochemical pregnancy				
	Positive		Negative		
Stimulation response	SOD N (%)	Placebo N (%)	SOD N (%)	Placebo N (%)	P
Number of follicles					
• ≥ 6	3 (75)	0 (0)	7 (43.8)	7 (36.8)	0.171
• < 6	1 (25)	1 (100)	9 (56.2)	12 (63.2)	
Total	4 (80)	1 (20)	16 (45.7)	19 (54.3)	
Endometrial thickness					
• < 10 mm	0 (0)	1 (100)	1 (6.2)	14 (73.7)	0.025
• ≥ 10 mm	4 (100)	0 (0)	15 (93.8)	5 (26.3)	
Total	4 (80)	1 (20)	16 (45.7)	19 (54.3)	

Table 5 Influence of Various Factors on Pregnancy Rates in Both Groups

	Biochemical pregnancy				
	Positive		Negative		
Factors	SOD N (%)	Placebo N (%)	SOD N (%)	Placebo N (%)	P
Age					0.171
<35 y	1 (25)	1 (100)	11 (68)	14 (73.7)	
≥35 y	3 (75)	0 (0)	5 (31.2)	5 (26.3)	
Sperm concentration					0.361
< 5 b/ml	2 (50)	1 (100)	8 (50)	14 (37.3)	
≥ 5 b/ml	2 (50)	0 (0)	8 (50)	5 (26.3)	
Indication					0.361
Sperm factor	2(50)	1(100)	8(50)	14(73.7)	
Unexplained	2(50)	0(0)	8(50)	5(26.3)	
Duration of infertility					
< 5 y	3 (75)	1 (100)	5 (31.2)	9 (47.4)	0.576
≥ 5 y	1 (25)	0 (0)	11 (68.8)	10 (52.6)	
Total	4 (80)	1 (20)	16 (45.7)	19 (54.3)	

natural and stimulated cycles, directing this study to do a comparison of endometrial thickness in both groups and its relevance to pregnancy rates. This study showed that four (80%) pregnancies in the SOD group occurred with an endometrial thickness of 10 mm or more and one pregnancy in the placebo group with endometrial thickness less than 10 mm. These results indicate that administration of antioxidants (SOD) significantly increased endometrial thickness (as a reflection of its receptivity) and, in turn, increased the pregnancy rate (confidence interval [CI] = 0.95, p = 0.025).

Other factors

The final success of assisted technology reproduction is largely determined by the success at each stage of the procedure. Therefore, we also compare the other variables that might influence the results, such as the age of the patient, sperm concentration, indication of insemination, duration, and type of infertility. As shown in Table 5, there were two pregnancies (40%) in women who were aged below 35 years in both the SOD group and the placebo group. However, all the three pregnancies of women aged above 35 years came entirely from SOD group. In this study, samples' heterogeneity was minimized by limiting the indication of insemination, that is, sperm concentration factors and unexplained infertility. Pregnancy was found to be 60% (n = 3) in infertility due to sperm factor and two pregnancy in unexplained infertility.

This study had shown that in the three pregnancies which were found on the sperm factor group, two (66.7%) of them originated from the SOD group, and two pregnancies in the unexplained infertility group came entirely from SOD group. Although these results were not statistically significant (p = 0.361), the pregnancy rates tend to be higher in the SOD group compared with the placebo group, either in sperm factor or in unexplained infertility. Similar results were found in sperm concentration group. Considering duration of infertility, in a group subjects with less than five years of infertility, three pregnancies were found in the SOD group and one in placebo group.

DISCUSSION

Effect of SOD on pregnancy rates

Although results were not statistically significant, this study suggested that the pregnancy rate tended to be higher in the group that was administered superoxide oxidase antioxidant. There is a lack of current research about the effect of SOD on the outcome of IUI. Thus, its role in other assisted reproductive procedures (e.g., *in vitro* fertilization [IVF], intracytoplasmic sperm injection [ICSI]) may be used as reference due to similarity in terms of procedure and process in ovarian stimulation, embryo implantation, and luteal phase defense. Agarwal et al.[8] reported that *in vivo* fertilization and embryo development occur in the milieu of low oxygen pressure. Therefore, during assisted reproductive procedure it is essential to avoid the conditions that trigger the production of ROS and their exposure to gametes and embryos. Low oxygen tension is more effective in increasing the implantation and pregnancy rate than higher oxygen tension. Similar to *in vitro* microenvironment, fertilization and pregnancy rate were higher with using culture media which supplemented antioxidants than with the use of standard culture media.[9–11] In multicenter randomized controlled trials, the effect of antioxidants in patients with luteal phase defect showed that pregnancy rate was higher in the treatment group compared to the control group. Similarly, low concentrations of antioxidants were significantly present in women with recurrent miscarriage and luteal phase defects than in healthy women.[2] A prospective case–control study revealed that SOD2 genotype is an important predictor to gaining pregnancy in IVF. The data also support the role of antioxidant defenses, particularly in mitochondrial mechanism of conception.[12]

Effect of SOD on ovarian stimulation responses

Synchronous development between endometrium and ovarian follicles are required in reproductive physiology to achieve pregnancy. Our study shows that the number of follicles increased by administration of SOD during ovarian stimulation, even though this finding was not statistically significant ($p = 0.075$). Whereas regarding endometrial development, SOD administration increased endometrial thickness and, in turn, increased pregnancy rates ($p = 0.025$). Alberto Revelli et al.[13] in a review concerning the correlation between biochemical markers and metabolomics of follicular fluid to oocyte quality outline that the supraphysiology level of ROS lead in the pathophysiology of embryonic development defects. Low level of ROS in follicular fluid was a potential marker for predicting the success of IVF procedure. Conversely, high level of ROS denotes various pathological conditions that affect fertility. Adverse effect of ROS can be impeded by minimizing free radicals or by using antioxidant enzymes such as SOD. TAC was found to be significantly higher in follicles containing oocytes with the ability to get fertilized than in follicles with oocytes that cannot be fertilized. TAC level can be used as a marker of mature follicles with good-quality oocytes. Furthermore, administration of 3 mg of melatonin per day as an antioxidant increases fertilization rates.[13] By contrast, an observational prospective study found that the activity of SOD was significantly higher in the follicular fluid of patients with unfertilized oocytes than that of patients with fertilized oocytes. It seems that SOD activity was varied and contrary with regards to the ability of oocyte in fertilization.[14] Regulation of the function of ovarian stromal and germ cell possesses a diverse pattern, and oxidative stress is one of the physiological regulators of stromal and germ cells. Granulosa and luteal cells in humans respond to hydrogen peroxide by ceasing gonadotropin activity and inhibiting progesterone secretion. Production of progesterone and estradiol was reduced when hydrogen peroxide was added in hCG-stimulated luteal cells. Hydrogen peroxide reduced steroidogenesis activity both in cAMP-dependent and non-cAMP-dependent pathways.[11]

The thickness of the endometrium is one of the clues that can be measured in practice to determine its receptivity. The results of this study support that endometrium is a tissue that physiologically and periodically undergoes process of angiogenesis. In normal or induced cycle, endometrium undergoes differentiation and involves angiogenesis and neovascularization. Both of these processes generate oxidative stress that involves the role of protein and FOXO3a mRNA and thus increase oxidative damage and apoptosis. Simultaneously, suppression of FOXO3a expression would cripple the signaling pathway that is responsible for the oxidative cell death. As a result, FOXO1 induces the expression of MnSOD in differentiated ESC. This suggests that endometrium has a defense mechanism against oxidative stress–induced defect through SOD activity. Cyclic changes in the endometrium are associated with the alteration of various antioxidants' expression. As well, the expression of SOD varies in a cyclic manner in the endometrium. In the late secretion phase, SOD activity was diminished whereas ROS level increased.[15] Follicles' development, selection of dominant follicle, corpus luteum formation, endometrial differentiation, and the formation of an embryo are the main processes that depend on neovascularization. In the development of endometrium, the spiral arteries and capillaries will be regenerated, and estrogen triggers angiogenesis in the endometrium by regulating the expression of growth factors such as vascular endothelial growth factor (VEGF). Angiogenesis is important for the cyclic regeneration of the endometrium during menstrual cycle. Cytokine complexes on feto-maternal surface play an important role in forming a proper micro environment that is necessary for implantation. ROS are produced in small quantities from growth factors and cytokines activated NADP(H)oxidase endothelial. ROS that emerged in or around the vascular endothelium play a role in normal cellular signaling mechanism and can be a factor that causes endothelial dysfunction that then disrupts perfusion and generates

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ischemia.[2,10] In terms of endometrial thickness, Kamath et al. in a prospective study on predictors of the success rates of IUI found that pregnancy rates tend to be higher in patients with endometrial thickness that is more than 6 mm.[16]

Role of other factors

It has been discovered that the relationship between age and reduction in the number and quality of follicles. Age-related oocyte quality affects many biochemical pathways that may interfere with the development of pre- and post-embryo implantations. The study of 40 women proves that alteration in granulosa cells attributed to ageing is mediated by oxidative stress. Along with ageing, decrement of mRNA that encodes SOD causes a decrease in its expression at the level of protein. Mitochondrial deterioration of granulosa cells and lipid depletion occurred during the reproductive senescence.[17] The chance of conception was enhanced with increasing sperm concentration by 40–50 million/mL. Traditionally, severe oligospermia is expressed as sperm concentration of less than 5 million/mL. Male infertility factors contribute to 20% of the overall causes of infertility while others report infertility factors in both partners contribute to 20–40% of reproductive failures.[18] In this study, we minimize the heterogeneity of samples with only sperm concentration abnormality and unexplained infertility selected as indications of IUI. Three pregnancies were obtained in the group of sperm factor, and two (66.7%) of them were entirely from the treatment group. Despite this result not being statistically significant, biochemical pregnancies tend to be higher in the SOD group than in placebo group, both in terms of sperm factor and unexplained infertility. Similar results were obtained with the study of sperm concentration, and pregnancies were more positive in the SOD group. Previous studies by Kamath et al.[16] had shown that the pregnancy rates of IUI was not significantly different based on the cause of infertility. Pregnancies were found higher in patients with unexplained infertility than those with infertility due to sperm factor; the results were not statistically significant, however.[16]

There are less data on the relationship between duration of infertility and achievement of IUI. Our study suggests that pregnancy rates seem to be lower in couples with lengthy duration of infertility. This is consistent with a prospective study by Kamath et al., which report that pregnancy rate was significantly lower with increasing duration of infertility.[16] Meanwhile, Fisch et al. used clomiphene citrate stimulations and reported that in the case of unexplained infertility cases, the pregnancy rate was 13% in those with infertility for 4.3 years and 14% in those with infertility for 2.5 years.[19]

CONCLUSION

This study suggests that administration of SOD in IUI program provides higher pregnancy rate than placebo even though the results were not significantly different. Antioxidant administration also improves response of ovarian stimulation and significantly increases endometrial thickness and then, subsequently, the pregnancy rate. The use of antioxidant, mainly SOD, seems beneficial in certain cases, including older women and cases of primary and recent infertility. A broader research is needed to arrive at a consensus about dose and duration of antioxidant administration. Further research is also necessary to observe the effect of antioxidant on ongoing pregnancy rate as well as in response to various methods of controlled ovarian hyperstimulation in assisted reproductive technology.

REFERENCES

1. Agarwal A, Gupta S, Razek HA, et al. Impact of oxidative stress on gametes and embryos in an *ART*1 laboratory. The Clinical Embriol 2007, 9(3):5–22.
2. Agarwal A, Gupta S, Sharma K. Role of oxidative stress in female reproduction. Reprod Biol and Endoc 2005, 3:28.
3. Oyawoye O, Gadir AA, Garner A, et al. Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: Relationship to outcome. Hum Reprod 2003, 18(11):2270–2274.
4. Sugino N, Harada AK, Sakata A, et al. Nuclear Factor-κB is required for tumor necrosis factor-α induced manganese superoxide dismutase expression in human endometrial stromal cells. J Clin Endocrin & Metabol 2002, 87(8):3845–3850.
5. Tatone C, Carbone MC, Dalone S, et al. Age-dependent changes in the expression of superoxide dismutases and catalase are associated with ultrastructural modifications in human granulosa cells. Mol Hum Reprod 2006, 12(11):655–660.
6. Ebisch IMW, Thomas CMG, Peters WHM, et al. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum Reprod 2007, 13(2):163–174.
7. Sugino N, Harada AK, Sakata A, et al. Different mechanisms for induction of copper-zinc superoxide dismutase and manganese superoxide dismutase by progesterone in human endometrial stromal cells. Hum Reprod 2002, 17(7):1709–1714.
8. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 2003, 79(4):829–843.
9. Koppenol WH. The Haber-Weiss Cycle—70 years later. Redox Report. W.S. Maney & Son Ltd 2001, 6(4):229–234.
10. Agarwal A. Oxidative stress and its implication in female infertility. Reprod Bio Med 2005, 11(5):641–650.
11. Puchner AM. Novel follicular fluid factors influencing oocyte developmental potential in IVF. Reprod Bio Med 2006, 12(4):500–506.
12. Ruiz-Sanz JI, Aurrekoetxea I, Matorras R, Ruiz-Larrea MB. Ala16Val SOD2 polymorphism is associated with higher pregnancy rates in in vitro fertilization cycles. Department of Physiology, Medicine and Dentistry School, University of Basque Country, Leioa, Spain. Fertil Steril 2011, 95(5):1601–1605.
13. Revelli A, Piane LD, Casano S, et al. Follicular fluid content and oocyte quality: From single biochemical markers to metabolomics. Department of Obstetrics, Gynecology,

and Reproductive Sciences, University of California, San Francisco, USA. Reprod Biol Endocrin 2009, 7:40. doi: 10.1186/1477-7827-7-40.
14. Sabatini L, Wilson C, Lower A, et al. Superoxide dismutase activity in human follicular fluid after controlled ovarian hyperstimulation in women undergoing in vitro fertilization. Fertil Steril 1999, 72(6):1027–1034.
15. Kajihara T, Jones M, Fusi L, et al. Differential expression of FOXO1 and FOXO3a confers resistance to oxidative cell death upon endometrial decidualization. Mol Endocrin 2006, 20(10):2444–2455.
16. Kamath MS, Bhave P, Aleyamma TK, et al. Predictive factors for pregnancy after intrauterine insemination: A prospective study of factors affecting outcome. J Hum Reprod Sciences 2010, 3(3):129–134.
17. Hawbe et al. Success of intrauterine insemination, in women aged 40-42 years. Fertil and Steril 2002, 78(1)
18. Fritz MA, Speroff L. Male infertility. Clinical gynecologic, endocrinology and infertility (8th ed.), 2011, pp.1250–1292.
19. Fisch P, Casper RF, Brown SE, et al. Unexplained infertility: evaluation of treatment with clomiphene citrate and human chorionic gonadotropin. Fertil Steril 1989, 51(5):828–833.
20. Agarwal A, Allamaneni SR. Artificial insemination. Infertility and Recurrent Pregnancy Loss 2007, 6(36):539–548.
21. Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. Current Opinion in Obstetrics and Gynecology 2006,18:325–332.
22. Das S, Chattopadhayay R, Ghosh S, et al. Reactive oxygen species level in follicular fluid embryo quality marker in IVF. Hum Reprod 2006, 21(9):2403–2407.
23. Frendo JL, Rond P, Bird T, et al. Overexpression of copper zinc superoxide dismutase impairs human trophoblast cell fusion and differentiation. Endocrinology. The Endocrine Society 2001, 142 (8):3638–3648.
24. Menvielle FJ. Superoxide dismutase (SOD), a powerful antioxidant, is now available orally. Pharmaceutical Sciences, Fabienne Joanny, Consulting & Licensing, Paris, France in Phytothérapie 2005-Numéro 3.
25. Sharma RK, Agarwal A. Role of reactive oxygen species in gynecologic diseases. Reprod Med Biol 2004, 3:177–199.
26. Tatemoto H, Muto N, Sunagawa I, et al. Protection of porcine oocytes against cell damage caused by oxidative stress during in vitro maturation: Role of superoxide dismutase activity in porcine follicular fluid. Biol Reprod 2004, 71:1150–1157.

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