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Oxidative Stress in Follicular Fluid of Low Responder and Hyporesponse Patients Undergoing the IVF Program at Halim Fertility Center, IVF Clinic, Division of Reproductive Endocrinology, Faculty of Medicine, USU Medan

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Abstract:-
Background: In vitro fertilization has not shown a significant increase in success rates, especially in low and hypo-responders. One of the causes is oxidative stress. This study aims to determine the relationship between level of ROS, TAC and ROS-TAC score in follicular fluid with oocyte maturity rate, oocyte quality rate, fertilization rate and embryo quality rate in low and hypo-responder patients.

Methods: An analytical observational study with a prospective cohort design conducted at Halim Fertility Center (HFC), Stella Maris Hospital, IVF Clinic, Division of Fertility Endocrinology Reproduction, Department of Obstetrics and Gynecology, USU Medical Faculty, Medan, from April 2021 until the number of samples is met.

Results: This study was followed by 84 patients. 33 patients from Poseidon 1 (P1) group, 30 patients from P2, 12 patients from P3 and 9 patients from P4. There was no significant difference based on the demographic characteristics of each group, except for age (p<0.001), duration of infertility and duration of stimulation (p<0.001 and p=0.004). The highest ROS level was in the P1 group with a mean of 462.92. There was a significant difference in the mean ROS levels between the four groups (p=0.049). There was no significant difference in TAC and ROS-TAC levels in the four groups (p=0.524) and (p=0.460). A significant correlation was found (r=0.458) between the TAC level and the rate of embryo quality in the P1 group (p=0.007). There was no significant difference in each poseidon group between ROS, TAC and ROS-TAC scores with oocyte maturity rate, oocyte quality rate, fertilization rate and embryo quality rate in hypo-responders.

Conclusion: ROS was significantly highest in the P1 group while TAC was significantly correlated with the rate of embryo quality also in the P1 group. Further research is needed to see the relationship between ROS, TAC and ROS-TAC score with oocyte quality, embryo and IVF success.

Keywords:- *In vitro fertilization, Hypo-responder, Low-responder, Oocyte, Oxidative stress, ROS, TAC, ROS-TAC score, POSEIDON.*

I. INTRODUCTION

Infertility is the inability to achieve pregnancy after one year of regular sexual intercourse without contraception. *The American Society for Reproductive Medicine* (ASRM) states that infertility is a disease, therefore early evaluation and treatment can be justified based on medical history, physical findings and in women over the age of 35 years after 6 months of marriage. [1.2]

Infertility problems can have a big impact. In addition to medical problems, infertility can also cause psychological and economic problems for couples who experience it. In fact, the overall incidence of infertility has remained relatively unchanged over the last few decades. However, the evaluation and treatment of infertility has changed dramatically over that time. [2.3]

These changes cannot be separated from the development of Assisted Reproductive Technology (ART). One form of this ART is in vitro fertilization (IVF). In vitro fertilization is a popular and widely accepted procedure for the treatment of infertility. Unfortunately, the success of IVF, which is measured as the average pregnancy rate per cycle, is only 30-40%. IVF failure causes a high treatment *dropout rate and is associated with the psychological condition of the partner.* [4–6]

Ovarian stimulation is an important step in IVF. Usually age and reduced ovarian reserve are associated with decreased efficacy of ovarian stimulation and will affect the success of IVF. [4–6] In most women performing conventional ovarian stimulation will result in adequate follicular growth and estrogen rates. The number of *mature* oocytes obtained is used as a parameter to see the ovarian response to exogenous *gonadotropins* so that the number of oocytes is closely related to live births in ART. [7]

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Poor responders account for 9–24% of patients undergoing ovarian stimulation in IVF and become one of the most difficult patient groups to treat in everyday infertility practice. This is due to: (1) Many studies were conducted in small numbers making it difficult to find differences between existing therapies, (2) The variety of definitions of *poor responders* reported in the literature, thus presenting a diverse group of patients, (3) Causes and mechanisms what causes POR is still unclear, especially in young women, (4) Differences in outcomes used in various studies to evaluate outcomes in these POR patients, (5) It is not possible to compare results from different studies due to large bias, (6) Limited level of multiple *meta-analyses* excludes many observational studies. [8.9]

In *poor responders* the mechanism of ovarian insufficiency is decisive and not well understood. Several causes of reduced ovarian reserve have been identified, including surgery on the ovaries especially in cases of endometriosis, genetic defects, chemotherapy, radiotherapy, autoimmune disorders, single ovary and smoking. Moreover, new risk factors for *low ovarian response*, namely diabetes mellitus type I, *transfusion-dependent - thalassemia*, uterine artery embolization for the treatment of uterine myomas. However, in most cases the mechanisms involved in follicle loss are not very clear. [9.10]

The role of this oxidative stress in female oogenesis and folliculogenesis is an area that requires further research. Scientific evidence shows that oxidative stress is an important mediator of conception, although there is a threshold level for the benefits and harms of oxidative stress. [11]

One of the causes of oxidative stress is *reactive oxygen species* (ROS). ROS are highly reactive molecules resulting from oxygen metabolism which can be free radicals and non-radicals.ROS are also produced continuously in the reproductive tract due to biochemical reactions such as those in the mitochondrial respiratory chain. ROS are physiologically important in the function of various cellular systems such as: (1) helping to fight infection, (2) intracellular signaling pathways, (3) male and female reproductive functions. However, if there is an imbalance between the production of ROS and antioxidants, oxidative stress will occur which causes structural and functional cellular damage such as: lipids, nucleic acids, carbohydrates, proteins and ultimately results in mitochondrial dysfunction, cell apoptosis and DNA damage. [12.13]

In male infertility, damage due to oxidative stress, especially related to sperm motility, has been observed since the 1940s. Currently, it is very clear that the oxidative stress is positively correlated with poor sperm parameters.According to Rakesh *et al.*, 1999, the ROS-TAC (*total antioxidant capacity*) score is a novel measure of oxidative stress and is superior to ROS or TAC alone in male infertility. What about female infertility, is there any effect of oxidative stress on oocyte development? [14.15]

Low follicular fluid ROS level, high follicular fluid TAC and high ROS-TAC scores were associated with the pregnancy cycle after ICSI[16] whereas according to S Das *et al*., 2006, and C Siristatidis*et al.*, 2016, follicular fluid ROS rates were associated with embryo quality in IVF. [17]However, there are still very limited studies on the effects of ROS on the reproductive system.[4]In addition, existing studies regarding ROS and antioxidant rates in follicular fluid related to oocyte and embryo development still provide conflicting data, and their effect on IVF results is not clear. [13]

Therefore, a new study is needed to see how the effect of oxidative stress on follicular fluid on the development, growth and quality of oocytes and embryos, especially in *low-responder* and *hypo-response patients*.

II. METHODS

A. Research design

This study is an analytical observational study with a prospective cohort design, to determine the relationship between ROS, TAC and ROS-TAC score in follicular fluid, with oocyte maturity rate, oocyte quality rate, fertilization rate and embryo quality rate in *low responder* and *hypo-response patients.*This research was conducted at the Halim Fertility Center (HFC), Stella Maris Hospital, IVF clinic, Division of Fertility Endocrinology Reproduction, Department of Obstetrics and Gynecology, USU Medical Faculty, Medan, from April 2021 until the number of samples was achieved.

B. Population

The target population is all *low responder* and *hypo-response patients* undergoing IVF program in Indonesia. The accessible population was *low responder* and *hypo-response patients* who underwent the IVF program at Halim Fertility Center (HFC), Stella Maris Hospital, IVF clinic, Division of Fertility Endocrinology Reproduction, Department of Obstetrics and Gynecology, USU Medical Faculty, Medan, during the study period and stated their willingness to participated in the study by signing an *informed consent form*.

C. Sample

The research subjects were the accessible population that fulfil the research criteria. The sampling method in this study was carried out by *consecutive sampling*, all subjects who came and fulfil the selection criteria were included in the study until the required number of subjects was achieved.

D. Subject Criteria

Subjects were selected according to their eligibility, namely *low responder* and *hypo-response patients*, who underwent the IVF program at Halim Fertility Center (HFC), Stella Maris Hospital, IVF clinic, Division of Fertility Endocrinology Reproduction, Department of Obstetrics and Gynecology, USU Medical Faculty, Medan and fulfil the criteria as follows:

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E. Inclusion Criteria

Inclusion criteria in this study were: age according to Poseidon criteria, groups 1 and 3: < 35 years, groups 2 and 4: ≥ 35years, BMI between 18.5-29.9 kg/m ^{2}, perform the IVF program for the initial time, absence of abnormalities of the reproductive tract assessed from medical record status, clinical examination, and hormonal examination, absence of the history of systemic disease, metabolic and endocrine disorders such as: heart, lung, liver, kidney, fever, autoimmune, hyperprolactin, thyroid, DM, PCOS and endometriosis, absence of the history of surgery on the reproductive system, not consuming alcohol more than 3 drinks/day, not smoking, not consuming caffeine more than 200 mg/day, not exercising more than 3-5 hours/day, not under stress, not taking certain drugs and not taking anti-oxidants in the last 3 months. For the low responder group Poseidon group 1 and 2: AFC 5, AMH 1.2 ng/ml. For low responder groups Poseidon group 3 and 4: AFC < 5, and AMH < 1.2 ng/ml.The patients wereundergoing the IVF program at Halim Fertility Center (HFC), Stella Maris Hospital, IVF clinic Division Fertilization of Reproductive Endocrinology, Department of Obstetrics and Gynecology, USU Medical Faculty, Medan, who was willing to participate in the study and signed the *informed consent form.*

F. Exclusion Criteria

Exclusion criteria were patients who refused to sign the *informed* consent form, and patients with damaged follicular fluid at the time of the study.

G. Procedure

- Patients who will undergo the IVF program at Halim Fertility Center (HFC), Stella Maris Hospital, IVF clinic, Division of Fertility Endocrinology Reproduction, Department of Obstetrics and Gynecology, USU Medical Faculty, Medan, based on the history, physical examination and supporting examinations (laboratory and ultrasound) obtained from interviews and medical records were adjusted according to the inclusion and exclusion criteria.
- Informed *consent was carried out*. Explained the purpose and benefits of the study and then asked the patient's consent to participate in the study.
- Patients underwent controlled ovarian stimulation procedures, both protocol agonists and protocol antagonists according to clinical conditions based on the Halim Fertility Center (HFC) SOP. Ovarian stimulation is started from day 2 or 3 of the menstrual cycle with the initial dose adjusted according to the patient's condition.
- When there are 3 follicles measuring 17mm, final oocyte maturation / egg breakdown with HCG is carried out and egg picking (OPU) is carried out 36 hours later.
- Follicular fluid aspiration is carried out by a FER consultant obstetrician who is certified to carry out the IVF program, using ultrasound and a single lumen 17G aspiration needle connected to a vacuum machine for follicular aspiration without flushing. Follicular fluid contaminated with blood was excluded.
- Certified embryologists performed enzymatic separation of cumulus and corona cells surrounding the oocyte (Hyase-10x, Vitrolife, Sweden) using a denuded pipette. Follicular fluid from each follicle obtained in each patient was combined and then centrifuged at 12000 g for 10-15 minutes and stored at -80 ^{oC} until examination.
- The denuded oocytes were assessed for maturity under a microscope at 40x magnification. Oocytes with Polar Body (PB) at the time of examination with a microscope after denudation and oocytes that released their Polar Body (PB) 4-6 hours after denudation were defined as mature oocytes. Then the oocyte maturation rate was calculated. The oocyte maturation rate was calculated by the formula: the percentage of mature oocytes compared to the total number of oocytes obtained.
- The oocytes were also seen for their morphology to assess their quality. Based on the morphology, oocytes are divided into oocytes with good morphology (good morphology) and oocytes with poor morphology (poor morphology). Then the oocyte quality rate was calculated using the formula: the percentage of oocytes with good morphology compared to the number of mature oocytes.
- In the mature oocytes, intracytoplasmic sperm injection (ICSI) was performed. The ICSI oocytes were placed in culture media (IVF, Vitrolife, Sweden) and placed in an incubator. Fertilization rate was assessed 16-18 hours after ICSI with the presence of a pronuclei (2PN) and two polar bodies (PB). Fertilization rate was assessed by the formula: percentage of the number of fertilized oocytes compared to the number of mature oocytes.
- The embryo with 2PN was observed for development until the 3rd day after ICSI. The quality of the embryo is seen from the rate of division. Normal embryo division was found to be more than equal to 4 cells on day 2 after ICSI under a microscope with 40x magnification. Then the embryos were divided into grades A, B, C, and D. Grades A and B embryos were grouped into good quality embryos and grades C and D into poor embryo quality. Then the quality rate of the embryos is calculated using the formula: percentage of good quality embryos compared to the total number of embryos. All these actions are performed by the same embryologist.
- Assessed the state of hypo-response in patients, namely by assessing the level of FOI (follicle to oocyte index). Based on this FOI level, the sample is divided into two; conditions of low ovarian sensitivity (FOI 50%) and normo ovarian sensitivity (FOI > 50%).
- Examination of follicular fluid ROS level using the ELISA method (Bioenzy BZ-08124253-EB).
- TAC examination of serum and follicular fluid using the ELISA method (Bioassay systems DTAC-100).
- The ROS-TAC scores were calculated, as follows: Both levels were normalized for distribution after converting ROS to log ROS + 1. Both log ROS + 1 and TAC were standardized to Z scores thus the variability were the same. The standard score was

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calculated by subtracting the control mean from the individual observation scores and dividing by the standard deviation of the control population.
- Furthermore, data processing and statistical tests were carried out.

H. Statistic analysis

After the data was collected, data verification, editing, and processing were carried outusing a computer program. In this study, the *p* level considered significant, was determined at <0.05 with a 95% confidence interval. The distribution of demographic data was processed by univariate analysis. The relationship between the two variables was processed using bivariate analysis. The results were reported in the form of a paper which was presented in front of the teaching staff of the Department of Obstetrics and Gynecology, USU Medical Faculty, Medan.

III. RESULTS

A. Demographic Characteristics of Research Subjects Low Responder

This study was followed by 84 patients who underwent the IVF program at *Halim Fertility Center* (HFC), Stella Maris Hospital, IVF clinic, Division of Fertility Endocrinology Reproduction, Department of Obstetrics and Gynecology, USU Medical Faculty. All subjects had fulfiled the inclusion criteria. Based on the results of the Poseidon criteria assessment, there were 33 subjects including the Poseidon group 1 (P1), 30 subjects in the Poseidon group 2 (P2), 12 subjects in the Poseidon group 3 (P3) and 9 subjects in the Poseidon 4 group (P4). The complete demographic characteristics of the subjects are presented in table 1.

The mean age at P1 and P3 was 31.27 years and 31.92 years in groups P2 and P4 with a mean age of 37.73 years and 38.44 years, respectively. The mean BMI in groups P1 and P3 were 23.36 kg/m ^{2} and 23.11 kg/m ^{2} while in groups P2 and P4 the mean BMI was 23.46 kg/m ^{2} and 24.74 kg/m ^{2}. There was no significant difference in mean BMI between the 4 study groups (p=0.534) based on the ANOVA test.

The highest rate of education in the four groups including higher education was 29 people (87.9%) in group P1, 25 people (83.3%) in group P2, 9 people (75%) in group P3, and 7 people (77 people). ,8%) in the P4 group. By using the Kruskal Wallis test, there were no differences in demographic characteristics based on the level of education in the four subject groups (p=0.565).

The most occupations in groups P1, P2 and P3 were housewives, which amounted to 10 people (30.3%) in group P1, 12 people (40%) in group P2, and 5 people (41.7%) in the P3 group. While in group P4 the most types of occupation were self-employed, which amounted to 3 people (33.3%). By using the Kruskal Wallis test, there were no differences in demographic characteristics based on the type of work in the four subject groups (p=0.667).

Characteristics Demographics	**P1 (n=33)**	**P2 (n=30)**	**P3 (n=12)**	**P4 (n=9)**	**P**
Age, years					
Average (SD)	31.27 (1.81)	37.73 (2.18)	31.92 (1.56)	38.44 (1.33)	<0.001 [a]
Median (min-max)	32 (28-34)	37 (35-43)	32 (30-34)	39 (36-40)	
BMI, kg/cm ^{2}					
Average (SD)	23.36 (2.52)	23.46 (2.8)	23.11 (2.81)	24.74 (3.14)	0.534 [b]
Median (min-max)	22.64 (18.73-29.05)	23.39 (18.82-29.14)	22.3 (19.63-28.17)	25.78 (20.31-29.69)	
Education, n (%)					
Low	0	2 (6.7)	0	0	0.565 [a]
Intermediate	4 (12,1)	3 (10)	3 (25)	2 (22.2	
High	29 (87.9)	25 (83.3)	9 (75)	7 (77.8)	
Occupation, n (%)					
BUMN/BUMD	2 (6,1)	1 (3,3)	0	0	0.667 [a]
Teacher/Lecturer	2 (6,1)	4 (13.3)	1 (8.3)	0	
Housewives	10 (30.3)	12 (40)	5 (41.7)	1 (11,1)	
Private sector employee	5 (15.2)	1 (3,3)	0	1 (11,1)	
Government Employees	5 (15.2)	5 (16.7)	2 (16.7)	1 (11,1)	
Health workers	5 (15.2)	3 (10)	3 (25)	2 (22.2)	
Military services	1 (3)	0	0	1 (11,1)	
Self-employed	3 (9.1)	4 (13.3)	1 (8.3)	3 (33.3)	
Causes of Infertility, n (%)					
Male Factor	14 (42.4)	5 (16.7)	4 (33.3)	4 (44.4)	0.093 [a]
Uterine Myoma	1 (3)	1 (3,3)	0	1 (11,1)	
Multiple Factor	8 (24.2)	8 (26.7)	5 (41.7)	1 (11,1)	
Poor Reserve	0	0	2 (16.7)	2 (16.7)	
Tubal Factor	4 (12,1)	6 (20)	1 (8.3)	1 (11,1)	
Unexplained Infertility	6 (18.2)	10 (33.3)	0	0	

Infertility duration, years					
Average (SD)	4.88 (2.21)	8.9 (3.55)	7.92 (2.61)	9.78 (3.9)	<0.001 [b]
Median (min- max)	5 (1-9)	9 (1-16)	7.5 (5-13)	10 (3-15)	
Stimulation duration, days					
Average (SD)	6.97 (0.81)	7.33 (1.32)	7.75 (0.97)	8.44 (1.24)	0.004 [a]
Median (min- max)	7 (6-9)	7 (4-11)	8 (6-9)	8 (7-11)	

Table 1: Demographic Characteristics of Research Subjects Low Responder

[a] *Kruskal Wallis,* [b] *Anova*

The most common causes of infertility in group P1 and group P4 were *male factors*, with 14 (42.4%) and 4 (44.4%), whereas in group P2, 10 people (33.3%) with*unexplained fertility* and 5 people in the P3 group with *multiple factors* (41.7%). There were no significant differences in characteristics based on causes of infertility in the four study groups after being analyzed using the Kruskal Wallis test (p=0.093).

The longest duration of infertility was indicated by group P4 with a mean of 9.78 years, and the shortest duration of infertility was group P1 with a mean of 4.88 years. Using the ANOVA test showed that there were differences in demographic characteristics based on the duration of infertility in the four study groups (p<0.001).

The longest duration of stimulation was shown by group P4 with a mean of 8.44 days, and the shortest duration of stimulation was group P1 with a mean of 6.97 days. Using the Kruskal Wallis test, it was shown that there were differences in demographic characteristics based on the duration of stimulation in the four study groups (p=0.004).

B. Differences in rFSH Dosage, Basal Antral Follicle Number, Preovulatory Follicle, Oocyte Number, MII Oocyte, Good Quality Oocyte, Fertilized Oocyte, Good Embryo Number, and Bad Embryo in Low Responder Subjects based on Poseidon Group

Table 2 presents the dose of rFSH, the number of basal antral follicles, preovulatory follicles, the number of oocytes, MII oocytes, good quality oocytes, fertilized oocytes, good and bad embryo countsin the four study groups. The highest dose of rFSH was in group P4 with a mean of 3000 IU, and the lowest dose of 1628.7 IU was in group P1. Using the Kruskal Wallis test, it was shown that there was a significant difference in the mean dose of rFSH between the four Poseidon groups (p<0.001).

The highest number of basal antral follicles was group P1 with an average of 14.27 and the lowest average number of basal antral follicles was group P4 with an average of 3.78. Using the Kruskal Wallis test, it was shown that there was a significant difference in the mean number of basal antral follicles between the four Poseidon groups (p<0.001).

The highest number of preovulatory follicles was in group P1 with an average of 23.12, and the lowest number of follicles was 9.44 in group P4. Using the Kruskal Wallis test showed that there was a significant difference in the mean number of preovulatory follicles between the four Poseidon groups (p<0.001).

The highest number of oocytes was group P1 with an average of 23.7 and the lowest mean number of oocytes was group P4 with an average of 8.11. Using the Kruskal Wallis test, it was shown that there was a significant difference in the mean number of oocytes between the four Poseidon groups (p<0.001).

The highest number of MII oocytes was group P1 with an average of 15.33 and the lowest mean number of MII oocytes was group P4 with an average of 5.44. Using the Kruskal Wallis test, it was shown that there was a significant difference in the mean number of MII oocytes between the four Poseidon groups (p<0.001).

The highest number of good quality oocytes was group P1 with an average of 9.09 and the lowest average number of good quality oocytes was group P4 with an average of 2.22. Using the Kruskal Wallis test, it was shown that there was a significant difference in the mean number of good quality oocytes between the four Poseidon groups (p<0.001).

The highest number of fertilized oocytes was group P1 with an average of 12.61 and the lowest mean number of fertilized oocytes was group P4 with an average of 3.89. Using the Kruskal Wallis test showed that there was a significant difference in the mean number of fertilized oocytes between the four Poseidon groups (p<0.001). The highest number of good embryos was group P1 with an average of 5.06 and the average number of good embryos was group P4 with an average of 1.33. Using the Kruskal Wallis test, it was shown that there was a significant difference in the mean number of good embryos between the four Poseidon groups (p<0.001). The highest number of bad embryos was group P1 with an average of 7.03 and the average number of bad embryos was group P4 with an average of 2.44. Using the Kruskal Wallis test, it was shown that there was a significant difference in the mean number of bad embryos between the four Poseidon groups (p<0.001).

Table 3 shows the results of the *posthoc* test (advanced test) from the different parameter tests contained in table 2. Based on the results of the analysis for rFSH doses, most showed that there was a significant mean difference between each Poseidon group. Only between groups P2 and P3 did not show a significant difference in mean dose (p=0.769) after being analyzed using the Independent T test.The results of the analysis for the number of basal antral follicles, mostly showed significant mean differences. However, between groups P1 and P2 (p=0.083) and between groups P3 and group P4 (p=0.754) there was no significant difference in the mean basal antral follicle count.

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The results of the analysis for the number of preovulatory follicles, almost all of them showed a significant mean difference. Only between group P3 and group P4 (p=0.625) did not show a significant difference in the mean number of preovulatory follicles. The results of the analysis for the number of oocytes, most of them showed a significant difference in mean. However, between groups P2 and P3 (p=0.105) and between groups P3 and group P4 (p=0.455) did not show a significant difference in the mean oocyte count.

The results of the analysis for the number of MII oocytes, mostly showed a significant difference in mean. However, between groups P2 and P3 (p=0.067) and between groups P3 and group P4 (p=0.490) there was no significant difference in the mean MII oocyte count. The results of the analysis for the number of good quality oocytes, most of them showed a significant mean difference. However, between groups P2 and P3 (p=0.051) and between groups P3 and group P4 (p=0.283) did not show a significant difference in the mean number of good quality oocytes.

The results of the analysis for the number of fertilized oocytes, most of them showed a significant mean difference. However, between groups P2 and P3 (p=0.128) and between groups P3 and P4 (p=0.386) did not show a significant difference in the mean number of fertilized oocytes. The results of the analysis for the number of embryos were good, most of them showed a significant mean difference. However, between groups P2 and P3 (p=0.070) and between groups P3 and group P4 (p=0.410) did not show a significant difference in the mean number of good embryos. The results of the analysis for the number of embryos were poor, most of them did not show a significant difference in the mean. However, between groups P1 and P3 (p=0.019) and between groups P1 and group P4 (p=0.011) showed a significant difference in the mean number of bad embryos.

Variable	**P1 (n=33)**	**P2 (n=30)**	**P3 (n=12)**	**P4 (n=9)**	**p***
rFSH dose, IU					
Average (SD)	1628.7 (322.35)	2108.33 (652.06)	2039.58 (750.72)	3000 (1057.64)	<0.001
Median (min- max)	1600 (1200-2725)	2087.5 (1200-3925)	1937.5 (1000-3975)	2575 (1650-4875)	
Basal Antral Follicle Number					
Average (SD)	14.27 (4.21)	12.37 (4.79)	3.83 (0.39)	3.78 (0.44)	<0.001
Median (min- max)	14 (7-20)	10.5 (6-20)	4 (3-4)	4 (3-4)	
Preovulatory Follicle Count					
Average (SD)	23.12 (0.3)	19.1 (12.85)	10.67 (6.67)	9.44 (3.58)	<0.001
Median (min- max)	21 (10-50)	15.5 (5-50)	9 (3-23)	9 (4-15)	
Oocyte Count					
Average (SD)	23.7 (13.41)	15.27 (10.22)	10.08 (7.97)	8.11 (3.48)	<0.001
Median (min- max)	19 (6-67)	12 (6-52)	10 (1-25)	8 (4-14)	
MII Oocyte Count					
Average (SD)	15.33 (8.66)	10.67 (7.65)	7 (5.92)	5.44 (3.4)	<0.001
Median (min- max)	15 (3-34)	8 (4-40)	5.5 (1-19)	4 (1-12)	
Good Quality Oocyte Count					
Average (SD)	9.09 (5.88)	6.27 (4.99)	3.42 (3,4)	2.22 (1,3)	<0.001
Median (min- max)	8 (1-26)	5.5 (1-21)	2.5 (0-11)	2 (1-5)	
Number of Fertilized Oocytes					
Average (SD)	12.61 (7.01)	8.4 (6.06)	5.67 (5.12)	3.89 (3.59)	<0.001
Median (min- max)	12 (2-27)	7 (2-30)	4 (0-16)	2 (0-11)	
Number of Good Embryos					
Average (SD)	5.06 (2.76)	3,2 (2,3)	1.92 (1.83)	1.33 (1.12)	<0.001
Median (min- max)	5 (0-11)	3 (0-12)	2 (0-5)	1 (0-3)	
Bad Embryo Count					
Average (SD)	7.03 (5.54)	5.07 (5.32)	3.33 (4.38)	2.44 (2.46)	<0.001
Median (min- max)	6 (0-22)	3 (0-26)	1 (0-14)	1 (0-7)	

Table 2: Differences in rFSH Dosage, Basal Antral Follicle Number, Preovulatory Follicle, Oocyte Number, MII Oocyte Number, Good Quality Oocyte Number, Number of Fertilized Oocytes, Number of Good Embryos, and Number of Bad Embryos by Poseidon Group

*Kruskal Wallis

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		P2	P3	P4
rFSH	P1	0.002 [a]	0.034 [a]	<0.001 [a]
	P2		0.769 [b]	0.038 [b]
	P3			0.036 [b]
Basal Antral Follicle Number	P1	0.083 [a]	<0.001 [a]	<0.001 [a]
	P2		<0.001 [a]	<0.001 [a]
	P3			0.754 [a]
Preovulatory Follicle Count	P1	0.021 [a]	<0.001 [a]	<0.001 [a]
	P2		0.022 [a]	0.014 [a]
	P3			0.625 [b]
Number of Oocyt	P1	0.001 [a]	0.001 [a]	<0.001 [a]
	P2		0.105 [a]	0.010 [a]
	P3			0.455 [b]
Number of Oocyt MII	P1	0.016 [a]	0.004 [b]	<0.001 [b]
	P2		0.067 [a]	0.015 [a]
	P3			0.490 [b]
Good Quality Oocyt Count	P1	0.024 [a]	0.003 [b]	<0.001 [b]
	P2		0.051 [a]	0.004 [a]
	P3			0.283 [b]
Numberof Fertilized Oocytes	P1	0.007 [a]	0.003 [b]	<0.001 [b]
	P2		0.128 [a]	0.012 [a]
	P3			0.386 [b]
Number of Good Embryos	P1	0.004 [a]	0.001 [b]	<0.001 [b]
	P2		0.070 [a]	0.009 [a]
	P3			0.410 [b]
Bad Embryo Count	P1	0.077 [a]	0.019 [a]	0.011 [a]
	P2		0.066 [a]	0.081 [a]
	P3			0.854 [a]

Table 3 Posthoc Test rFSH Dose, Basal Antral Follicle Number, Oocyte Number, MII Oocyte Number, Good Quality Oocyte Number, Number of Fertilized Oocytes, Number of Good Embryos, and Number of Bad Embryos by Poseidon Group

[a] Mann Whitney, [b] T Independent

C. Differences in FOI, ROS, TAC, and ROS TAC Score based on Poseidon Group on Low Responder Subjects based on Poseidon Group

Table 4 presents the levels of FOI, ROS, TAC, ROS TAC Scorein the four study groups. The highest FOI level was in group P3 with an average of 255.56, and the lowest level of 124.31 was in group P2. Using the Anova test showed that there was a significant difference in the mean FOI level between the four Poseidon groups (p=0.002). By categorizing the FOI level, 2 subjects (6.1%) in group P1 and 2 subjects (6.7%) in group P2, and 2 people (16.7%) in group P3 with FOI level 50% or called the hyporesponse group.

The highest ROS level was in the P1 group with a mean of 462.92, and the lowest level of 355.99 was in the P3 group. Using the Kruskal Wallis test, it was shown that there was a significant difference in the mean ROS levels between the four Poseidon groups (p=0.049). The highest TAC level was in group P4 with an average of 627.64, and the lowest level was 551.6 in group P1. Using the Kruskal Wallis test, it was shown that there was no significant difference in the mean TAC level between the four Poseidon groups (p=0.524).

The highest ROS TAC level was in group P3 with an average of 52.92, and the lowest level of 47.81 was in group P1. Using the Anova test, it was shown that there was no significant difference in the mean ROS TAC level between the four Poseidon groups (p=0.460).

The highest level of oocyte maturity rate was in group P3 with an average of 74.13, and the lowest level of 64.6 was in group P1. Using the Kruskal Wallis test, it was shown that there was no significant difference in the mean level of the oocyte maturity rate between the four Poseidon groups (p=0.364). The highest oocyte quality rate was in group P1 with a mean of 60.75, and the lowest level was 42.69 in group P3. Using the Anova test, it was shown that there was no significant difference in the mean level of the oocyte quality rate between the four Poseidon groups (p=0.227).

The highest level of the fertilization rate was in group P1 with a mean of 82.89, and the lowest level of 65.74 was in group P4. Using the Kruskal Wallis test, it was shown that there was no significant difference in the mean level of the fertilization rate between the four Poseidon groups (p=0.413). The highest level of embryo quality rate was in group P1 with a mean of 46.35, and the lowest level of 33.93 was in group P3. Using the Kruskal Wallis test, it was shown that there was no significant difference in the mean level of the embryo quality rate between the four Poseidon groups (p=0.318).

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Table 5 shows the *posthoc test results* (advanced test) of the FOI and ROS variables contained in table 4. Based on the results of the analysis for the FOI level, most showed that there was no significant difference in the mean between each Poseidon group. Only between groups P1 and P2 showed a significant difference in the mean FOI level (p=0.044) after being analyzed using the Tamhane test.

The results of the analysis for the level of ROS, mostly indicate a mean difference which was not significant. However, between group P2 and group P4 (p=0.033) and between group P3 and group P4 (p=0.019) demonstrated a significant difference in the mean ROS levels.

Variable	**P1 (n=33)**	**P2 (n=30)**	**P3 (n=12)**	**P4 (n=9)**	**P**
FOI					
Average (SD)	172.9 (84.82)	124.31 (50.98)	255.56 (195.86)	225.93 (125.17)	0.002 [a]
Median (min- max)	162.5 (30-350)	126.67 (50-273.68)	250 (25-625)	200 (100-466.67)	
50%	2 (6,1)	2 (6.7)	2 (16.7)	0	0.782 [b]
> 50%	31 (93.9)	28 (93.3)	10 (83.3)	9 (100)	
ROS					
Average (SD)	462.92 (197.08)	382.07 (104.75)	355.99 (111.47)	449.19 (99.09)	0.049 [c]
Median (min- max)	411.78 (199.48-961.03)	350.7 (253.36-639.98)	325.45 (272.68-691.94)	467.46 (294.82-642.18)	
TAC					
Average (SD)	551.6 (230.65)	566.46 (288.77)	564.01 (246.71)	627.64 (188.45)	0.524 [c]
Median (min- max)	445.4 (270.48-1108.18)	521.73 (203.19-1520.31)	459.29 (351.13-1190.8)	607.77 (387-1059.8)	
ROS TAC Score					
Average (SD)	47.81 (10.08)	51.44 (9.42)	52.92 (9.03)	49.39 (7.05)	0.460 [a]
Median (min- max)	49.88 (27.63-70.4)	52.34 (32.79-79.55)	51.1 (39.05-73.36)	46.86 (38.29-60.21)	
Oocyte Maturity Rate					
Average (SD)	64.6 (17.18)	71.06 (19.29)	74.13 (23.62)	65.21 (24.97)	0.364 [c]
Median (min- max)	62.5 (27.27 – 100)	73.22 (36.36 – 100)	76.52 (24 – 100)	75 (25 – 100)	
Oocyte Quality Rate					
Average (SD)	60.75 (26.82)	58.46 (24.93)	42.69 (34.36)	50.46 (26.88)	0.227 [a]
Median (min- max)	63.64 (17.86 – 100)	58.57 (16.67 – 100)	45 (0 – 100)	50 (12.5 – 100)	
Fertilization Rate					
Average (SD)	82.89 (13.83)	78.04 (15.29)	71.77 (29.03)	65.74 (32.39)	0.413 [c]
Median (min- max)	82.14 (50 – 100)	80 (40 – 100)	79.17 (0 – 100)	66.67 (0 – 100)	
Embryo Quality Rate					
Average (SD)	46.35 (23.31)	42.71 (26.89)	33.93 (36.55)	34.72 (30.58)	0.318 [c]
Median (min- max)	42.86 (0 – 100)	45.3 (0 – 100)	21.59 (0 – 100)	30 (0 – 100)	

Table 4: Differences in FOI, ROS, TAC, ROS TAC Score, Oocyte Maturity Rate, Oocyte Quality Rate, Fertilization Rate, and Embryo Quality Rate based on Poseidon Group on Low Responder Subjects

[a] *Anova,* [b] *Mann Whitney,* [c] *Kruskal Wallis*

		P2	**P3**	**P4**
FOI	P1	0.044 [a]	0.699 [a]	0.833 [a]
	P2		0.225 [a]	0.227 [a]
	P3			0.999 [a]
ROS	P1	0.127 [b]	0.061 [b]	0.490 [b]
	P2		0.344 [b]	0.033 [b]
	P3			0.019 [b]

Table 5: Posthoc FOI and ROS assays by Poseidon Group

[a] *Tamhane,* [b] *Mann Whitney,* [c] *T Independent*

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Table 5 presents the results of the analysis of the relationship between rates of ROS, TAC, ROS TAC Score with oocyte maturity rate, oocyte quality rate, fertilization rate (fertilization) and embryo quality rate in low responder subjects based on the Poseidon group. In general, no significant relationship was found between rates of ROS, TAC, ROS TAC Score with oocyte maturity rate, oocyte quality rate, fertilization rate (fertilization) and embryo quality rate (p>0.05) in each Poseidon group. Only in group P1 there was a significant correlation between the TAC score and the rate of embryo quality (p = 0.007) with a correlation level (r) = 0.458, meaning that there was a moderate strength correlation between the TAC Score and the rate of embryo quality.

		P1 (n=33)		**P2 (n=30)**		**P3 (n=12)**		**P4 (n=9)**	
		P	**r**	**p**	**r**	**p**	**r**	**p**	**r**
ROS	**Oocyte Maturity Rate**	0.461 [a]	-0.133	0.548 [a]	-0.114	0.511 [a]	0.211	0.511 [a]	0.211
	Oocyte Quality Rate	0.061 [a]	-0.333	0.214 [a]	0.234	0.076 [a]	-0.530	0.739 [b]	-0.108
	Fertilization Rate	0.627 [a]	-0.088	0.081 [a]	-0.324	0.576 [a]	-0.180	0.735 [b]	-0.109
	Embryo Quality Rate	0.943 [a]	0.013	0.320 [a]	-0.188	0.293 [a]	0.331	0.858 [b]	0.058
TAC	**Oocyte Maturity Rate**	0.526 [a]	-0.114	0.823 [a]	0.043	0.120 [a]	-0.474	0.431 [a]	0.301
	Oocyte Quality Rate	0.347 [a]	-0.169	0.598 [a]	0.100	0.065 [a]	0.548	0.858 [b]	-0.070
	Fertilization Rate	0.232 [a]	0.214	0.952 [a]	-0.011	0.568 [a]	-0.183	0.690 [b]	0.155
	Embryo Quality Rate	**0.007 [a]**	**0.458**	0.070 [a]	0.335	0.293 [a]	0.331	0.791 [b]	-0.103
ROS TAC Score	**Oocyte Maturity Rate**	0.463 [b]	-0.132	0.372 [b]	0.169	0.208 [b]	-0.392	0.559 [a]	0.226
	Oocyte Quality Rate	0.444 [b]	0.138	0.813 [b]	0.045	0.149 [b]	0.443	0.994 [b]	0.003
	Fertilization Rate	0.222 [a]	0.219	0.203 [a]	0.239	0.767 [b]	0.096	0.564 [b]	0.223
	Embryo Quality Rate	0.275 [b]	0.196	0.124 [b]	0.287	0.708 [a]	0.121	0.368 [b]	-0.342

Table 6: Relationship between ROS, TAC and ROS-TAC Score in Follicular Fluid with Oocyte Maturity Rate, Oocyte Quality Rate, Fertilization Rate and Embryo Quality Rate in Low Responder Subjects based on Poseidon Group

[a] *Spearman,* [b] *Pearso*

D. Relationship between ROS, TAC and ROS-TAC scores in follicular fluid with oocyte maturity rate, oocyte quality rate, fertilization rate and embryo quality rate in hyporesponder subjects (FOI 50%)

There were only 6 subjects included in the hyporesponse, with a total of 2 people from group P1, group P2 and group P3 each. None of the subjects included in the hyporesponse of group P4 of the 84 subjects who participated in this study.

Table 7 shows the relationship between rates of ROS, TAC and ROS-TAC Score in follicular fluid with oocyte maturity rate, oocyte quality rate, fertilization rate and embryo quality rate in Hyporesponder Subjects. From the results of the analysis using the correlation test showed that there was no significant correlation between the rates of ROS, TAC and ROS-TAC Score in follicular fluid with the rate of oocyte maturity, oocyte quality rate, fertilization rate and embryo quality rate in Hyporesponder Subjects (p>0.05).

		Hyporesponder Subject (n=6)	
		P	**r**
ROS	**Oocyte Maturity Rate**	0.263 [a]	-0.546
	Oocyte Quality Rate	0.109 [b]	0.717
	Fertilization Rate	0.594 [b]	-0.278
	Embryo Quality Rate	0.468 [b]	0.372
TAC	**Oocyte Maturity Rate**	0.454 [b]	-0.383
	Oocyte Quality Rate	0.109 [b]	0.717
	Fertilization Rate	0.954 [b]	0.031
	Embryo Quality Rate	0.069 [b]	0.778
ROS TAC Score	**Oocyte Maturity Rate**	0.652 [a]	-0.236
	Oocyte Quality Rate	0.338 [b]	0.478
	Fertilization Rate	0.355 [b]	0.463
	Embryo Quality Rate	0.069 [b]	0.778

Table 7: Relationship between ROS, TAC and ROS-TAC Score in Follicular Fluid with Oocyte Maturity Rate, Oocyte Quality Rate, Fertilization Rate and Embryo Quality Rate in Hyporesponder subjects (FOI 50%)

[a] *Pearson,* [b] *Spearman*

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Relationship of Age, BMI, Duration of Infertility and Duration of Stimulation with ROS, TAC and ROS-TAC Scores in Follicular Fluid in Low Responder Subjects based on Poseidon Group

Table 8 presents the results of the analysis of the relationship between age, BMI, duration of infertility and duration of stimulation with rates of ROS, TAC, ROS-TAC Score in low responder subjects based on the Poseidon group. In the three study groups, namely groups P1, P2 and P4 showed no significant relationship was found between age, BMI, duration of infertility and duration of stimulation with rates of ROS, TAC, ROS-TAC Score ($p > 0.05$). On the other hand, in the P3 group, there was a significant correlation between the duration of infertility with the ROS level and the ROS-TAC score ($p<0.05$).

By using the Spearman correlation test, we found a significant correlation between the duration of infertility with the ROS level ($p = 0.002$). The correlation level obtained is 0.786, meaning that there was a positive correlation with a strong correlation between the duration of infertility and the ROS level, meaning that the greater the duration of infertility, the higher the ROS level will be.

Furthermore, a significant correlation was also found between the duration of infertility and the ROS-TAC score ($p = 0.012$). The correlation level obtained is -0.693, meaning that there is a negative correlation with a strong correlation between the duration of infertility and the ROS-TAC score, meaning that the greater the duration of infertility, the lower the ROS-TAC score.

		P1 (n=33)		P2 (n=30)		P3 (n=12)		P4 (n=9)	
		P	**R**	**p**	**r**	**p**	**r**	**p**	**r**
Age	**ROS**	0.313 [a]	0.181	0.499 [a]	0.128	0.791 [a]	-0.086	0.873 [b]	-0.062
	TAC	0.799 [a]	-0.046	0.367 [a]	0.171	0.895 [a]	-0.043	0.095 [b]	-0.589
	ROS-TAS Score	0.799 [a]	-0.046	0.613 [a]	0.096	0.633 [a]	-0.154	0.308 [b]	-0.384
BMI	**ROS**	0.374 [a]	-0.160	0.077 [a]	0.328	0.542 [a]	0.196	0.994 [b]	-0.003
	TAC	0.547 [a]	-0.109	0.566 [a]	0.109	0.354 [a]	-0.294	0.592 [b]	-0.208
	ROS-TAS Score	0.758 [a]	0.056	0.415 [a]	-0.154	0.208 [a]	-0.392	0.681 [b]	-0.160
Duration of Infertility	**ROS**	0.878 [a]	0.028	0.989 [a]	0.003	**0.002 [a]**	**0.786**	0.268 [b]	-0.414
	TAC	0.328 [a]	0.176	0.790 [a]	0.051	0.533 [a]	-0.200	0.790 [b]	0.104
	ROS-TAS Score	0.222 [a]	0.219	0.711 [a]	0.070	**0.012 [a]**	**-0.693**	0.354 [b]	0.351
Stimulation Length	**ROS**	0.371 [a]	-0.161	0.471 [a]	-0.137	0.626 [a]	0.157	0.591 [b]	-0.208
	TAC	0.245 [a]	0.208	0.319 [a]	0.188	0.092 [a]	-0.508	0.480 [b]	-0.272
	ROS-TAS Score	0.113 [a]	0.281	0.173 [a]	0.255	0.174 [a]	-0.420	0.783 [b]	-0.107

Table 8: Relationship of Age, BMI, Infertility and Stimulation Duration with ROS, TAC and ROS-TAC Score in Follicular Fluid in Low Responder Subjects based on Poseidon Group

[a] *Spearman*, [b] *Pearson*

IV. DISCUSSION

Reactive Oxygen Species (ROS) are oxidizing compounds in the form of oxygen and its derivatives which are highly reactive and unstable, which disrupt many types of cell molecules such as lipids, proteins, and nucleic acids (DNA).[18,19]ROS are formed during normal cellular metabolism. ROS has a role like a double-edged sword. ROS are involved in many physiological processes, but excessive amounts can also cause pathological processes, especially in the female reproductive tract. The presence of ROS in the female reproductive tract has been confirmed by many studies.[20]

Physiologically, ROS influence early embryonic development, affecting implantation and fertilization of the egg. Pathologically, ROS itself can cause fertility problems for women.Among infertile women, one of the main causes of low IVF success rates is related to impaired oocyte growth and maturation resulting in poor embryo quality and decline. The success characteristics of IVF have been found to be related to ROS rates and antioxidant capacity of follicular fluid during oocyte retrieval.[19] The study also focused on a group of patients with *low prognosis* based on Poseidon criteria.[21,22]

The results showed that in general there was no significant difference between TAC rates and ROS-TAC Score ($p>0.05$) in each Poseidon group. However, it was found that the highest ROS levels were significantly different in the P1 group ($p=0.049$). It might be expected that ROS rates in the P4 group were the highest due to ROS production which generally increases with age. However, the findings in this study indicated that the increase in the level of ROS was influenced by many factors other than age (multifactorial). [21,22]

Furthermore, in this study, in general, there was also no significant difference between oocyte maturity rate, oocyte quality rate, fertilization rate, and embryo quality rate among the four Poseidon groups ($p>0.05$). The same thing was also found in the FOI levels in the four poseidon groups.[23] In this study, in general, there was no significant relationship found between rates of ROS, TAC, ROS-TAC Score with oocyte maturity rate, oocyte quality rate, fertilization rate (fertilization) and embryo quality rate

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(p>0.05) in eachPoseidon group. Only in the P1 group, there was a significant correlation between the TAC level and the rate of embryo quality (p=0.007) with a correlation level (r)=0.458, meaning that there was a moderate strength correlation between the TAC score and the quality rate of the embryo.

V. CONCLUSION

This study was followed by 84 low responder patients who underwent the IVF program at Halim Fertility Center, Stella Maris Hospital, Medan. Based on the Poseidon criteria, the sample was divided into 4 groups, namely33 people from Poseidon 1 group (P1), 30 people from Poseidon group 2 (P2), 12 people from Poseidon group 3 (P3) and 9 people from Poseidon 4 group (P4). There were no significant differences based on the demographic characteristics of each group, except for: age where the mean age in groups P1 and P3 was 31.27 years and 31.92 years and in groups P2 and P4 was 37.73 years and 38.44 years, the longest duration of infertility was indicated by group P4 with a mean of 9.78 years and the shortest duration of infertility was group P1 with a mean of 4.88 years, the longest duration of stimulation was indicated by group P4 with an average of 8.44 days and the shortestduration of stimulation was group P1 with a mean of 6.97 days.

Based on the FOI level of $\leq$ 50%, only 6 subjects were included in the hyporesponse with a total of 2 people each from group P1, group P2 and group P3. None of the subjects included in the hyporesponsefrom group P4 in this study.There were significant differences (p=<0.001) dose of rFSH, number of basal antral follicles, number of preovulatory follicles, number of oocytes, number of MII oocytes, number of good quality oocytes, number of fertilized oocytes, number of good embryos and number of bad embryos from each group.The highest ROS level was in group P1 with a mean of 462.92 and the lowest was in group P3 with a mean of 355.99. A significant difference in mean ROS levels was found between the four groups (p=0.049). The highest TAC level was in group P4 with an average of 627.64 and the highest ROS-TAC level was in group P3 with an average of 52.92, while the lowest TAC and ROS-TAC levels were in group P1 with an average of 551.6 and 47.81. There was no significant difference in TAC and ROS-TAC levels in the four groups (p=0.524) and (p=0.460).

From the follow-up tests for ROS levels in the four groups, between groups P2 and P4 (p=0.033) and groups P3 and P4 (p=0.019) showed a significant difference in the mean ROS levels.In this study, in general, no significant relationship was found between rates of ROS, TAC and ROS-TAC score with oocyte maturity rate, oocyte quality rate, fertilization rate and embryo quality rate (p>0.05) in each Poseidon group. Only in the P1 group there was a significant correlation between the TAC level and the rate of embryo quality (p=0.007) with a correlation level (r) of 0.458, meaning that there was a moderate strength correlation between the TAC level and the quality rate of the embryo.

REFERENCES

[1.] Bayer S R, Alper M M, Penzias A S. The Boston IVF handbook of infertility: A practical guide for practitioner who care for infertile couples. 3th ed. Informa healthcare. 2012.

[2.] Hendarto H, Wiweko B, Harzif A K, et al. konsensuspenangananinfertilitas. Ed 2. Hiferi – POGI. Jakarta. 2019.

[3.] Speroff L, fritz M A. Clinical gynecologic endocrinology and infertility. 8th ed. Lippincott Williams and wilkins. USA. 2011.

[4.] Das S, Chattopadhyay R, et al. Reactive oxygen species in follicular fluid – embryo quality marker in IVF?. Human reproduction journal. 2006. 2403 – 07.

[5.] Cologne R N, Cortes S, Gonzales M R G, et al. Oxidative stress in follicular fluid of young women with low response compared with fertile oocyte donors. Elsevier. 2015. 10.1016.

[6.] Conforti A, et al. Management of Women with an Unexpected Low Ovarian Response to Gonadotropin. Frontiers. 2019, Juni. 10.3389.

[7.] Alviggi C, et al. Understanding Ovarian Hypo-response to Exogenous Gonadotropin in Ovarium Stimulation and Its New Proposed Marker-The Follicle to Oocyte (FOI) Index. Frontiers. 2018, Oktober. 10.3389.

[8.] Polyzos NP, et al. Management Strategy For Poseidon Group I. Frontiers. 2019. Oktober. 10.3389.

[9.] Ubaldi F, et al. Management of Poor Responders in IVF: Is There Anything New? Hindawi. 2014. 10.1155.

[10.] Drakopoulos P, et al. Update on the Management of Poor Ovarian Response in IVF: The Shift From Bologna Criteria to the Poseidon Concept. The Adv Reproduction Health. 2020. 10.1177.

[11.] Agarwal A, et al. Oxidative Stress in Applied Basic Research and Clinical Practice. Springer. New York. 2013. DOI: 10.1007. (4): 75-95, (12): 205-36.

[12.] Karabulut S, et al. Effects of Follicular Fluid Oxidative Status on Human Mural Granulosa Cells, Oocyte Competency and ICSI Parameters. Elsevier. 2020. 10.1016.

[13.] Agarwal A, et al. Oxidative Stress In Human Reproduction . Springer. 2017. DOI: 10.1007. (1): 1-17.

[14.] Armstrong D, Stratton RD. Oxidative Stress and Antioxidant Protection. Ed 1. New Jersey. Wiley Blackwell. 2016. (15): 221-52

[15.] Sharma RK, et al. The Reactive Oxygen Species-Total Antioxidant Capacity Score is a New Measure of Oxidative Stress to Predict Male Infertility. Human Reproduction. 1999. 2801-2807.

[16.] Bedaiwy MA, et al. Effect of Follicular Fluid Oxidative Stress Parameters on Intracytoplasmic Sperm Injection Outcome. Informa. 2012. 10.3109.

[17.] Siristatidis C, et al. The Effect of Reactive Oxygen Specieson Embryo Quality in IVF In Vivo. 2016. (30): 149-154

[18.] Abdul-Razzaq LN, et al. Evaluation of Total Antioxidant Capacityin Serum and Follicular Fluid of Women Undergoing ICSI and Its Association with

Implantation Failure. Medico Legal Update. 2020. Vol. 20. No. 2.
[19.] Appasamy M, et al. Evaluation of the Relationship Between Follicular Fluid, Oxidative Stress, Ovarian Hormones, and Response to Gonadotropin Stimulation. Fertility Sterility. Elsevier. Vol. 89. No. 4. 10. 1016.
[20.] Du Plessis SS, et al. Impact of Oxidative Stress on IVF. Expert Reviews Ltd. 2008. 10.1586.
[21.] Olszak-Wasik K, et al. From "Every Day" Hormonal to Oxidative Stress Biomarkers in Blood and Follicular Fluid, to Embry Quality and Pregnancy Success? Hindawi. 2019. 10.1155.
[22.] Attaran M, et al. The Effect of Follicular Fluid Reactive Oxygen Species on the Outcome of In Vitro Fertilization. The Crateand Clinic Foundation. 2000.
[23.] Khazaei M, Aghaz F. Reacive Oxygen Species Generation and Use of Antioxidants during In Vitro Maturation of Oocytes. Royan Institute. International Journal of Fertility and Sterility Vol. 11. No.2. 2016.