Effects of Gnrh Agonist and Gnrh Antagonist Stimulation Protocols on Oxidative Stress Status in Follicular Fluid of Women Undergoing IVF: Results from A Large, Prospective, Single-Center Analysis

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Abstract

Objective: The aim of our study was to evaluate the impact of GnRH agonist and GnRH antagonist stimulation protocols on the level of oxidative stress in the follicular fluid of women who are undergoing in vitro fertilization (IVF). Our goal was to help clinicians choose the most suitable protocol that causes minimal oxidative stress while achieving successful IVF outcome.

How stimulation technique affect Lipid Peroxidation Malondialdehyde (MDA), Trolox Equivalent Antioxidant Capacity (TEAC), Super Oxide Dismutase (SOD), and Catalase in follicular fluid and IVF results in 102 women who underwent In Vitro Fertilization (IVF) at the Center of Obstetrics and Genecology IBN ROCHD Constantine, Algeria. During ovum pick-up, follicular fluid was collected to evaluate oxidative stress indicators such as SOD, Catalase, MDA, and TEAC.

The subjects who received the antagonist protocol had higher levels of SOD than those who received GnRHa protocols in the GnRHa group, the mean concentrations of FFSOD(3,60± 1,50 UI/ml/mg Protein vs 4,48± 1,17UI/ml/mg Protein; p<0.002), Catalase (2,03± 1,35 vs 2,99±2,00 U/mg Protein; p =0.015) .

In GNRH-a, the mean concentration of FF MDA (1, 09±0, 51 nMole/l vs0, 87±0, 36 nMole/l; p=0,008).

Compared to the other group, the antagonist protocol had the lowest average level of MDA.

In comparison to the antagonist protocol, the GnRHa protocol had a higher mean TEAC value.

The results showed that the changes in MDA were significant, but they were much lower in the antagonist groups compared to the agonist protocol, with a statistical significance of p=0.008

Conclusion:

The study has provided evidence that altering the protocol of ovarian stimulation leads to a variation in the level of oxidative stress parameters. The antagonist groups had significantly lower levels of MDA than those following the agonist protocol

Keywords: Follicular fluid, GnRH agonist protocol (GnRHa), GnRH antagonist protocol, IVF/ICSI, Oxidative stress.

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Introduction

Millions of people around the globe suffer from infertility, and the present treatment options have a success rate of only 40%, leaving out important information. Gerris et al., « Prevention of Twin Pregnancy after In-Vitro Fertilization or Intracytoplasmic Sperm Injection Based on Strict Embryo Criteria »..

The primary objective of IVF is to provide a full-term, live birth and to improve the chances of conceiving healthy children for infertile individuals.

In order to produce more embryos for selection and transfer, the ovarian stimulation technique aims to stimulate the growth of many follicles and oocytes.

Numerous variables influence the outcome of Assisted Reproductive Technology (ART), including the ovarian stimulation protocols and oxidative stress.

Long-acting gonadotrophin-releasing hormone agonists (GnRHa) were first used in ovarian stimulation in ART in the late 1980's to suppress natural pituitary gonadotropin production and to avoid an exogenous gonadotropin-induced luteinizing hormone (LH) surge. Ludwig et al., « Tailoring the GnRH Antagonist Cetrorelix Acetate to Individual Patients' Needs in Ovarian Stimulation for IVF ».. In the long protocol, GnRHa is administered during the midluteal phase, which triggers a reaction that releases stored pituitary gonadotropins. On the other hand, GnRH antagonists are preferred over long-acting agonists as they competitively and dose-dependently block the GnRH receptor without causing flares. Unlike agonists, antagonists require daily injections to prevent an early LH surge. Moreover, antagonists are more effective in inhibiting endogenous gonadotropin secretion.

The process of ovarian stimulation is associated with the production of Reactive Oxygen Species (ROS) and the disturbance of the balance between oxidants and antioxidants, which can impact

oxidative stress markers and the success of IVF procedures. Indeed, accumulating evidence suggests that ROS are required for proper fertilization, The balance between ROS (reactive oxygen species) and antioxidants plays a crucial role in achieving reproductive success both in vivo and in vitro. Shiotani et al., « Immunohistochemical Localization of Superoxide Dismutase in the Human Ovary »; Suzuki et al., « Superoxide Dismutase in Normal Cycling Human Ovaries »..Despite the growing attention about this topic, the available pieces of evidence does not allow to draw a firm conclusion about the topic.

In this scenario, we designed a prospective observational study to investigate whether there is a variation in the oxidative condition of the follicular fluid for women who are infertile and undergoing in vitro fertilization (IVF) while following two distinct protocols - agonists and antagonists of luteinizing hormone-releasing hormone (LMA GNRH). The task involves selecting a suitable protocol that results in a lower level of oxidative stress.

Materials and Methods

We prospective enrolled consecutive women scheduled for IVF at the Centre of Obstetrics and Genecology IBN ROCHD Constantine, Algeria, from October 2020 to January 2021. Every participant who was a part of this research project provided their consent to gather and analyze their data for research purposes.

The ethics committee of the Ibn Rochd clinic has given its approval for the study.

We considered eligible infertile women who met the following inclusion criteria:

The inclusion criteria for this study comprised individuals undergoing their first IVF cycle, who were less than 35 years old, had a basal follicle stimulating hormone (FSH) of 10 mIU/mL, a basal metabolic index (BMI) of 19e30 kg/m2, and had no ovaries-related disorders either currently or in the past. Endometriosis cases and poor responders were excluded.

During the study, demographic information was gathered through an interview using a pre-designed and pre-tested questionnaire. Women undergoing IVF treatment were assigned to one of two protocols: either the GnRH agonist or the GnRH antagonist protocol.

The study involved seventy patients who were subjected to mild ovarian stimulation with the GnRH antagonist cetrorelix (Cetrotide®, Serono) and recombinant FSH (Gonal F®, Serono). The rFSH cycle began on the fifth day, with a dosage of 150 IU if the antral follicle count (AFC) was greater than 10, and 225 IU if the AFC was less than 10. Once the largest follicle reached a diameter of 14 mm, a subcutaneous dose of 0.25 mg/day of cetrorelix was administered.

In this study, Thirty two patients were given the standard long GnRH agonist treatment using leuprolide acetate 1 mg/day s.c (Lucrin®, Abbot,) starting on day 21 of their previous menstrual

cycle. on the 3rd day of the cycle, the dose of leuprolide was decreased to 0.5 mg/day, and a daily dosage of rFSH was started at either 225e300 or 375 IU, taking age and BMI into consideration.

The patients were monitored through transvaginal ultrasound scans. If more than 2 follicles larger than 18 mm were detected, a 250 mg human chorionic gonadotropin (hCG) injection was given to facilitate final oocyte maturation. After 36 hours, ovum pick-up (OPU) was carried out, and single embryo transfer (SET) was performed three days later. The luteal phase was maintained with 8% vaginal progesterone until the pregnancy test, which was conducted 12 days after the embryo transfer. Ultrasound observation of fetal heartbeats between 7-8 weeks of gestation confirmed clinical pregnancy. The classification of oocyte maturity and embryo grading was based on Veeck et al's guidelines.« Veeck, L. (1990) The Morphological Assessment of Human Oocytes and Early Conception. In Keel, B.A. and Webster, B.W., Eds., Handbook of the Laboratory Diagnosis and Treatment of Infertility, CRC Press, Boca Raton, 353-369. - References - Scientific Research Publishing »..

On cycle day 3, all patients had their serum levels of anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), thyroid-stimulating hormone (TSH), and estradiol (E2) checked before beginning IVF treatment.

The study's main finding was the variation in FF-TEAC,FF SOD,FF Catalase ,FF MDA levels between the groups. The difference in pregnancy rates across the groups was a secondary finding of our research.

The participants were then classified into two classes depending On the stimulation protocol (GnRH agonist and GnRH antagonist)

Sample preparation

During the process of oocyte retrieval, for each patient, follicular fluid (FF) from mature follicles larger than 17 mm was aspirated and collected. To prevent any contamination from erythrocytes and leukocytes, the fluids were spun at 800g for 10 minutes. The resulting supernatant was cautiously collected and stored at a temperature of -80 C until just before the experiments mentioned in the following section.

Determination of enzymatic antioxidants

SOD assay

To measure the activity, the pyrogallol autoxidation was inhibited using a UV/vis spectrophotometer at 420 nm for three minutes, as previously explained in [7]. The enzyme activity was quantified as IU/ml/mg protein, where 1U corresponds to the enzyme amount that

results in a 50% decrease in pyrogallol autoxidation. SOD activity is defined as the amount of enzyme necessary to achieve a 50% reduction in pyrogallol auto-oxidation per ml of assay mixture.

Catalase assay

To measure the activity, a spectrophotometric method [8] was used. The reduction of H2O2 at 240 nm was observed on a UV/vis spectrophotometer at a temperature of 25°C. The enzyme activity that resulted in the decomposition of 1 mmol of H2O2 in one minute was considered as one unit of activity. The specific activities were denoted as IU/ml/mg protein.

Thiobarbituric Acid Reactive Substances (TBARS) assay for lipid peroxidation measurement

The measurement of lipid peroxidation was done by utilizing Thiobarbituric Acid Reactive Substances (TBARS) assay. This method involved the detection of substances that reacted with TBARS, with MDA being a by-product of the lipid peroxidation process. The reaction between MDA and TBA in a ratio of 2:1 resulted in a pink chromogen, which was then quantified using a spectrophotometer. To perform the assay, trichloroacetic acid, TBA, and hydrochloric acid were added to 0.5 ml of FF solution, and the mixture was homogenized before being subjected to a water bath at 100 C for 15 minutes. The tubes were then cooled and centrifuged, and the supernatant was collected and transferred to a microplate for further analysis. The quantification of TBARS was done using a spectrophotometer at a wavelength of 532 nm, and the results were compared to a standard.de Lima et al., « Follicular Fluid Lipid Peroxidation Levels in Women with Endometriosis during Controlled Ovarian Hyperstimulation »..

Determination of ABTS radical scavenging capacity

The ABTS radical scavenging capacity of follicular fluid was evaluated by Pellegrini Re et al., « Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay »..

To produce the ABTS radical solution, 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate were reacted overnight. The solution was then diluted with ethanol until an absorbance of 0.700 at 734 nm was achieved. The serum's total antioxidant capacity was measured using the ABTS scavenging capacity method. The ABTS+ solution (0.70 _ 0.01 OD at 734 nm) was mixed with 10 µL of serum (diluted 1/10 v/v in H20), and the mixture was incubated for 10 minutes. The results were compared to a standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) , an analog of vitamin E, and expressed in mmol Trolox equivalent L-1. The ABTS levels were also expressed in mmol Trolox equivalent/l of sample.

Statistical analysis

Statistical analysis of the clinical data was performed through SPSS 21.0 software (SPSS Chicago, IL, USA).

Using the Shapiro-Wilk test, a normal distribution was shown to exist. Means, standard deviations, medians, frequencies, and percentages are used to express data. For independent samples, non-normally distributed data were analyzed using the Mann-Whitney U-test, while normally distributed data were analyzed using the independent-sample ttest. Using χ 2with Yates correction, patients' categorical characteristics were compared. To investigate the connection between FF-TAC, FF SOD, FF Catalase, FF MDA and gonadotropin total dose, Pearson correlation was performed. . A p-value < 0.05 was considered significant.

Results

Table 1 Demographic data with respect to ovarian stimulation protocols.

	GnRH agonist group (n=32)	**GnRH antagonist group (n=70)**	***P***
Age (years)	31,56±2,70	30,44±3,42	**0,105**
BMI,kg/m2	27,75± 3,99	27,25± 3,79	**0.54**
Primary infertility (%)	22 (68,75%)	50 (69,44%)	**0,82**
Secondary infertility (%)	10(31,25%)	22 (30,56%)	**0,45**
Infertility duration (years)	6,84±4,20	6,97±4,09	0,89
Mean oocyte retrieved	7,28± 4,59	7,46±5,13	**0.869**
Baseline day 3 serum FSH in (mIU/ml)	5.99 ±1.02	4.85± 0.99	**0.0001***
Baseline day 3 serum LH in (mIU/ml)	3,46±0,95	4,33±1,38	**0,002***
Baseline day 3 serum E2 in (pg/ml)	55,77±19,74	56,99±29,57	0,832
Baseline day 3 serum TSH in (mIU/ml)	1,76±0,93	2,95±2,06	0,108

Baseline day 3 serum Prolactine in (ng/ml)	22.61±13,43	16,97±8,81	0,109
Baseline day 3 serum AMH in (ng/ml)	2,41±2,75	2,80±2,56	0,675
No. of total follicles on hCG day	7,09±4,41	8,24±4,77	0.25
Gonadotropin starting dose (IU)	268,75±70,99	241,43±59,27	0,045 *
Total dose of Gonadotropin-analogue (IU)	2602,35±802,55	2309,41±608,72	0,045 *
Duration of stimulation (days)	10,14± 1,75	8,88 ±2,15	0,052 *
Serum E2 levels on hCG day in pg/ml	2000,31±1017,51	1946,63±971,57	0.79
Endometrial thickness in mm on hCG day	10.44±2.18	11.07±2.69	0.6

AMH: anti-müllerian hormone; BMI: body-mass index; E2: estradiol; FSH: follicle stimulating hormone; LH: luteinizing hormone; TSH: thyroid stimulating hormone; PRL: prolactin,

Data presented as means ± standard deviation or number (%). *p < 0.05 is significant.

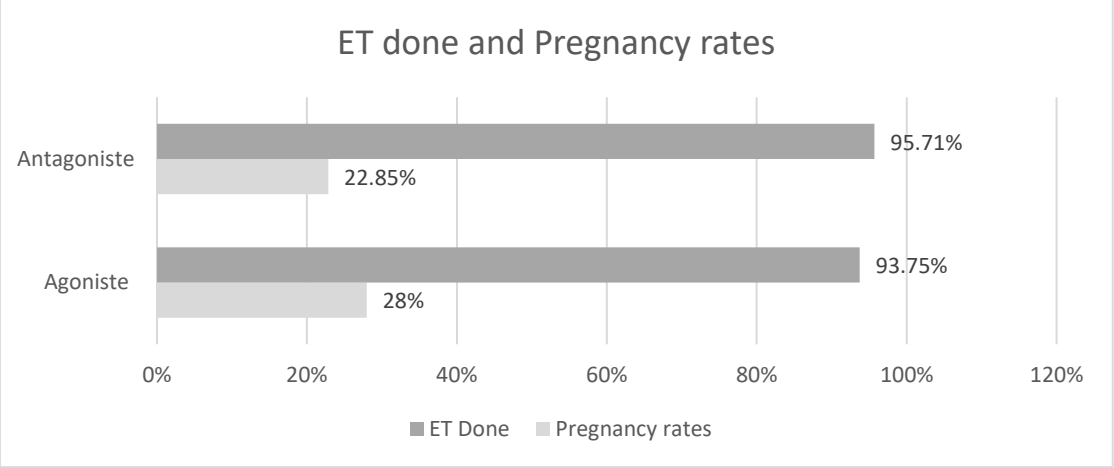
Table 2.Comparison of the laboratory and embryologic data between groups.

	GnRH agonist group(n=32)	**GnRH antagonist group(n=70)**	***P value***
Number of oocyte retrieved	7,28±4,59	7,46±5,1	0,87
Number of MII oocytes	5,69±4,27	5,30±3,58	***0,63***

Number of ICSI performed oocytes	5,09±4,09	**4,84**±3,79	***0,763***
Number of pronuclear (PN) zygote 2	2,34±2,46	**2,69**±2,76	***0,55***
Number of grade 1 embryo on day 3	3,56±2,45	3,70±2,68	***0,80***
Fertilization rate (%)	51,23%	**57,51%**	***0,46***
Pregnancy rate (%)	28,12%	**22,86%**	***0,57***

Data presented as means ± standard deviation, or number (%). *p < 0.05 is significant.

The fertilization rate (%) defined as the ratio between the number of fertilized oocytes at 2PN and the number of mature oocytes injected.



ET: Embryos transferred

Fig 1.ET done and positive pregnancy rates with respect to protocol used.

Table3. Oxidative stress with respect to ovarian stimulation protocol.

	GnRH agonist group(n=32)	**GnRH antagonist group(n=70)**	***P value***
Superoxydedismutase U/ml/mg Protein	3,60± 1,50	4,48± 1,17	***0,001***

Catalase U/mgProteine	2,03± 1,35	2,99±2,00	***0,021***
MDA nMole/l	1,09±0,51	0,87±0,36	***0,008***
TEAC(ABTS)mMole/L	1,21±0,29	1,19±0,3	**0.87**

TEAC: Troloxequivalentantioxidantcapacity,MDA:Manodialdhyde.Data presented as means ± standard deviation, . *p < 0.05 is significant

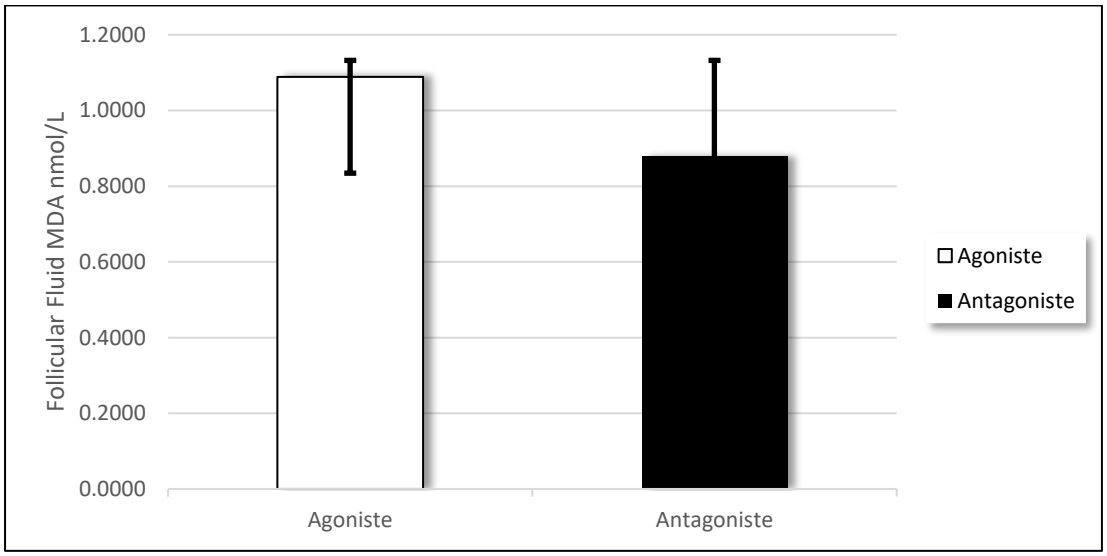


Fig. 2. FF-TAC levels among groups.

Table4. Comparison of the Oxidative stress for pregnant and not pregnant subgroups.

	Pregnant patients of the agonist group (n:9)	Pregnant patients of the antagonist stimulation group (n:16)	*P*
Age (years)	31,33±2,29	30,31±3,75	***0,34***
BMI (kg/m2)	26,89±1,09		***0,31***

		28,03±4,49	
Infertility duration (years)	6,56±3,40	8,44±3,58	***0,26***
Day 3 serum FSH (mIU/ml)	5,79±1,15	5,08±1,04	***0,088***
Day 3 serum E2 (pg/ml)	50,11±16,96	62,65±19,64	***0,71***
Day 3 serum AMH (ng/ml)	3,29±1,41	3,25±1,67	***0,92***
Serum E2 level on the day of HCG (pg/ml)	2226,22±968,41	2168,89±921,76	***0,81***
Total gonadotropin dose (rFSH) (IU)	1250 (659-1913)	1464 (1324-2081	***0,004****
Duration of stimulation (days)	9,56±2,60	8,60±2,074	***0,14***
Follicular fluid Superoxydedismutase U/ml/mg Protein	4,88±0,97	4,34±1,09	***0,23***

Follicular fluid Catalase U/mgProteine	2,83±1,12	3,34±2,69	***0,60***
Follicular fluid MDA nMole/l	1,26±0,77	0,79±0,24	***0,031****
Follicular fluid TEAC(ABTS)mMole/L	1,36±0,18	1,28±0,28	***0,48***
Number of oocyte retrieved	10,89±5,71	9,63±4,33	***0,61***
Number of ICSI performed oocytes	8±6,06	5,94±3,60	***0,53***
Number of pronuclear (PN) zygote 2	3,44 ±4,24	3,25 ±2,90	***0,49***
Number of Embryos transferred	2,22±0,44	2,81±1,22	***0,098***

Results

Demographic data is given in Table 1. The antagonist mild group had considerably lower gonadotropin starting dose, total dose, and stimulation duration in days.

Oocyte and embryo quality did not significantly differ across the groups. Table 2

The number of pregnant patients was 16 in the antagonist mild group (16/70, 22.85%) and it was 9 in the long agonist group (9/28, 13%), the rates of fertilization did not differ significantly between the groups.

The pregnant subgroups did not differ in terms of follicular fluid parameters, except for MDA, which is significantly different for the two groups, Compared to the Pregnant patients of the agonist group , the antagonist protocol had the lowest average level of MDA.

Further, However, we did discover a marginally favorable correlation between FF-TAC and total gonadotropin dose (r = 0.232, p = 0.019).

Ovarian stimulation, oxidative stress and IVF outcome

Subjects who underwent the antagonist protocol had higher levels of SOD in comparison to those who underwent GnRHa protocols, in the GnRHa group, the mean concentrations of FFSOD(3,60± 1,50 UI/ml/mg Protein vs 4,48± 1,17UI/ml/mgProtein; p<0.002), Catalase (2,03± 1,35 vs 2,99±2,00 U/mg Protein; p =0.015) .

In GNRH-a, the mean concentration of FF MDA (1,09±0,51 nMole/l vs0,87±0,36 nMole/l; p=0,008).

Compared to the other group, the antagonist protocol had the lowest average level of MDA.

The mean TEAC was higher in the GnRHa protocol compared to antagonist (Table 2).

The protocol used in this study played a significant role in the observed changes in MDA levels. As the study results indicate, the changes that were statistically significant for MDA were significantly lower in the antagonist groups (p=0.008) as compared to the agonist protocol. Therefore, it is important to carefully consider the choice of protocol when conducting research on MDA levels. A well-designed protocol can help ensure that accurate and reliable data is obtained, enabling researchers to draw meaningful conclusions.

Finally, the highest Embryo Transfer (ET) rate was observed with the antagonist protocol group, but the proportion of positive IVF outcome was slightly lower in this group.

It is noteworthy that the choice of protocol can significantly impact the success rates of IVF procedures. As evidenced by the study results, the agonist protocol group demonstrated a notable 30% positive IVF outcome, showcasing the efficacy of this particular protocol. This highlights the importance of carefully selecting the appropriate protocol to ensure the best possible outcome for patients undergoing IVF treatments.

Discussion

The ART treatment procedure's success is influenced by a number of factors, including the ovarian stimulation protocol.

The procedure used is determined by a variety of parameters, including age, baseline hormonal state, number of antral follicles, prior IVF, and others.

The aim of this study was to evaluate a potential correlation among different ovarian stimulation techniques, oxidative stress indicators in the follicular fluid, and IVF success.

In contrast to the antagonist group, which had less gonadotropin and fewer stimulation days, our findings showed that the agonist group had comparable oocyte quality, pregnancy rates.

When compared to the antagonist group, the agonist group follicular fluid MDA levels were greater.

Additionally, we discovered a favorable correlation between the total gonadotropin dose and FF-MDA. These results confirm our theory that elevated levels of exogenous gonadotropin cause an increase in oxidative stress.

Patients with low ROS indicators had a higher percentage of deliveries, whereas those with higher ROS had a higher incidence of miscarriages

A study have found link between oxidative stress and the ovarian stimulation protocol after ovarian stimulation Tulić et al., « Oxidative Stress Markers in GnRH AgonistaAnd Antagonist Protocols in IVF ».: these study did find a substantial lower level of serum SOD and a significantly higher amount of serum MDA.

However, the value of SOD, catalase groups differs significantly among patients with different stimulation protocols. Contrary to the results reported Aurrekoetxea et al., « Serum Oxidizability and Antioxidant Status in Patients Undergoing in Vitro Fertilization »..

MDA measurements differ significantly among patients with different stimulation protocols (p=0,047).

Exogenous FSH was given during the mid-tolate follicular phase by the use of mild ovarian protocols to prolong the FSH gate.

After ovarian stimulation, Tulic et al. found a substantially lower level of serum SOD and a significantly greater amount of serum MDA and sulfhydryl groupsTulić et al..

Higher FF TEAC level are associated with pregnancy after ICSI., Bedaiwy et al corroborates the findings of the study Bedaiwy et al. . It has been demonstrated that serum concentrations of MDA were lower in the GnRH antagonist group (long/short protocol) compared to the GnRH agonist group, which is comparable to our findings, but SOD was higher in the GnRH antagonist group, which is contradictory to the findings of this studyCelik et al., « A Comparative Study on Oxidative and Antioxidative Markers of Serum and Follicular Fluid in GnRH Agonist and Antagonist Cycles »..In GNRH agonists, the FF concentration of SOD and catalase activity was low, but in GNRH antagonists, the FF concentration of SOD and catalase activity was high.

These findings support the hypothesis, brought forward in this study, of an increase in oxidative stress due to increased amounts of exogenous gonadotropin.

In this study, the stimulation periods did significantly vary significantly between the groups. Although the total gonadotropin dosage (rFSH) differed significantly between the groups in the study at hand, pregnancy rates were similar, which is consistent with the literature.

The number of recovered oocytes, as well as the quantity and quality of oocytes/embryos, were not significantly different across the groups when using fresh single embryo transfer. In both fresh and

thawed IVF cycles,Casano et al found that the moderate stimulation regimen resulted in identical pregnancy ratesCasano et al., « MILD ovarian stimulation with GnRH-antagonist vs. long protocol with low dose FSH for non-PCO high responders undergoing IVF »..

There are several protocols that have been suggested to address the issue of supraphysiological levels of progesterone and oestrogen in the luteal phase of IVF cycles. These protocols aim to mitigate the potential adverse effects of these hormonal imbalances on the endometrium and oocyte/embryo. With careful consideration and implementation of such protocols, it may be possible to improve the success rates of IVF cycles. Kolibianakis et Devroey, « The Luteal Phase after Ovarian Stimulation ».Ertzeid, « The impact of ovarian stimulation on implantation and fetal development in mice »..

Increased morphological anomalies have been observed in oocytes when these are exposed to high gonadotropin doses during in vitro maturationRoberts et al., « Follicle-Stimulating Hormone Affects Metaphase I Chromosome Alignment and Increases Aneuploidy in Mouse Oocytes Matured in Vitro1 ».Van Blerkom et Davis, « Differential Effects of Repeated Ovarian Stimulation on Cytoplasmic and Spindle Organization in Metaphase II Mouse Oocytes Matured in Vivo and in Vitro »..

In another study mild stimulation resulted in fewer oocytes and a decreased proportion of aneuploid embryosBaart et al., « Milder Ovarian Stimulation for In-Vitro Fertilization Reduces Aneuploidy in the Human Preimplantation Embryo ».Munne et al., « Treatment-Related Chromosome Abnormalities in Human Embryos »..

Further, different ovarian stimulation protocols resulted in different rates of mosaicism in good-quality embryos.

Developing a protocol for measuring oxidative stress (OS) in the reproductive system is crucial in understanding how it affects fertility. With OS contributing to numerous physiological and pathological conditions in the ovary, it is essential to establish a standardized procedure that can measure OS levels accurately. By creating a protocol that accounts for variations in ROS and antioxidant levels, healthcare professionals can better diagnose and treat conditions related to reproductive health.Ruder, Hartman, et Goldman, « Impact of oxidative stress on female fertility »..OS was shown to increase in repeated ovarian stimulation leading to mitochondrial DNA mutation and a decrease in oocyte quality Chao et al., « Repeated Ovarian Stimulations Induce Oxidative Damage and Mitochondrial DNA Mutations in Mouse Ovaries ».. Van Blerkom and Davis demonstrated the effect of repeated ovarian stimulation in mice, resulting in a significant increase in the frequency of spindle defects resulting in chromosomal errors with each consecutive series of ovarian stimulationVan Blerkom et Davis, « Differential Effects of Repeated Ovarian

Stimulation on Cytoplasmic and Spindle Organization in Metaphase II Mouse Oocytes Matured in Vivo and in Vitro »..

Further, another study suggested that the methylation of imprinted genes in embryos was altered by gonadotropins in a dose dependent manner Market-Velker et al., « Dual Effects of Superovulation ».. Those findings lead us to investigate the follicular fluid anti oxidative capacity in two different IVF treatments.

The individuals, who were kept on agonist protocol, had the highest pregnancy rates. The decreased pregnancy rate with antagonist protocol might be due to greater oocyte numbers at retrieval, which could lead to implantation failure in a new IVF cycle and an increased risk of Ovarian Hyperstimulation Syndrome, as described.Celik et al., « A Comparative Study on Oxidative and Antioxidative Markers of Serum and Follicular Fluid in GnRH Agonist and Antagonist Cycles ».,Celik et al did find that the number of embryos at 2PN in the GnRH agonist stimulation is higher compared to GnRH antagonist stimulation in a statistically significant matter.

The formation of many follicles may result in the creation of supraphysiological steroid hormone levels, which may affect implantation, placentation, and perhaps neonatal outcome Steward et al., « Oocyte Number as a Predictor for Ovarian Hyperstimulation Syndrome and Live Birth ».. In addition, Lai et al showed that antagonist protocols resulted in greater implantation and clinical pregnancy rates than agonist protocols, whileShresta at al found that antagonist protocols resulted in higher implantation and clinical pregnancy rates than agonist protocols these results are controversialLai et al., « Comparison of the GnRH agonist and antagonist protocol on the same patients in assisted reproduction during controlled ovarian stimulation cycles ».Shrestha, La, et Feng, « Comparison of Different Stimulation Protocols Used in in Vitro Fertilization: A Review ».

ROS are regulatory mediators in signaling pathways at low concentrations, but they are detrimental at larger ones.

Endometriosis cases were not included in this analysis. The long agonist group's mean FF-TAC levels were higher than those obtained in the antagonist mild group. This disparity could be due to gonadotropin dosage, as more exogenous gonadotropins can generate increased OS, necessitating more antioxidant capability to counteract this response. This study also discovered a link between the two.

Despite its findings, the present report still has certain limitations that must be considered. Firstly, the study design was observational in nature, and as such, confounding variables like age could not be entirely eliminated during the recruitment of patients who were undergoing different GnRH analogue regimens. Additionally, the FF was collected from only a dominant follicle, which may not be an accurate representation of other follicles in the ovary. Although protocol was followed,

it is important to note that FF specimens of a single follicle may not be sufficient to indicate granulosa cell production.

Conclusion:

Our study confirmed that there is a difference in the concentration of oxidative stress parameters when changing the protocol of ovarian stimulation.

The antagonist groups had significantly lower levels of MDA, higher levels of Superoxide dismutase and catalase than those following the agonist protocol.

The number of oocytes collected is not associated with a significant change in the parameters of oxidative stress or the outcome of AMP procedures.

However, the results of IVF are better in patients using the agonist protocol while the number of embryos transferred is better for the antagonist protocol.

The pregnant subgroups did not differ in terms of follicular fluid parameters, except for MDA, which is significantly different for the two groups, Compared to the Pregnant patients of the agonist group , the antagonist protocol had the lowest average level of MDA.

Further, However, we did discover a marginally favorable correlation between FF-TAC and total gonadotropin dose ($r = 0.232$, $p = 0.019$).

Conflict of interest Statement

All authors have nothing to disclose.

AUTHOR CONTRIBUTION STATEMENT

AH, KB, MK, AZ, LO, SB, AR, LR conceived the study and wrote the paper. AH, KB, MK, AD, MB reviewed the article before publication performed experiments and analyzed data.

AH, MZ, KB Realized the statistical part of the study.

AL, AR reviewed the article before publication.

L,R thesis director.

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