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Synergistic Impact of Follicular TNF- α and Growth Hormone on Oocyte Competence and Pregnancy Success in Assisted Reproduction

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ABSTRACT: Background

Inflammatory and endocrine factors play essential roles in regulating ovarian physiology and reproductive outcomes. Tumor necrosis factor- α (TNF- α), a pro-inflammatory cytokine, and growth hormone (GH), a key endocrine regulator, are involved in follicular development, steroidogenesis, and cellular differentiation within the ovary. The aim of this study to evaluate the association of TNF- α and GH levels in serum and follicular fluid with ovarian response, oocyte and embryo characteristics, and pregnancy outcomes in women undergoing ICSF.

Methods

This prospective study included 120 infertile women undergoing ICSI, categorized into poor, normal, and hyper responders. Serum and follicular fluid levels of TNF- α and GH were measured at oocyte retrieval day (ORD) and embryo transfer day (ETD). Associations with hormonal profiles, oocyte characteristics, embryo quality, and pregnancy outcomes were assessed using non-parametric statistical methods.

Results

Both TNF- α and GH levels differed significantly across ovarian response groups ($p < 0.001$), with higher levels observed in hyper responders. TNF- α demonstrated significant positive correlations with key hormonal parameters and oocyte characteristics, particularly total oocyte count and maturation indices. GH showed similar associations with oocyte quantity and maturity, although follicular fluid GH exhibited negative correlations with MII oocytes.

Despite these biological associations, neither TNF- α nor GH showed consistent relationships with pregnancy outcomes. However, TNF- α demonstrated moderate predictive value, with a cutoff ≤ 185.32 at ETD yielding an AUC of 0.778, sensitivity of 84.6%, and specificity of 64.7%.

Conclusion

TNF- α and GH are significantly associated with ovarian activity and oocyte development in ICSI patients, reflecting their roles in inflammatory and endocrine regulation of the follicular microenvironment. However, their predictive value for pregnancy outcomes remains limited, suggesting that additional factors contribute to implantation success.

INTRODUCTION

Successful outcomes in assisted artificial reproductive technology ART, especially through ICSI procedures, involves a complex interplay among a series of tightly regulated processes of endocrine signaling, immunomodulation, and regulation by follicular microenvironment [1]. Although conventional indicators for success in ART procedures and especially in ICSI have mainly included the use of endocrine factors such as follicle-stimulating hormone (FSH), estradiol, and anti-Müllerian hormone (AMH), emerging scientific data show that these alone cannot be considered sufficient explanations for variation in ovarian reserve, ovarian response, oocyte competency, and implantation efficiency. In this context, there is an effort to identify other biological mediators that might impact ovarian function and pregnancy outcome [2,3].

The ovarian follicle constitutes an extremely complex environment where endocrine, paracrine, and autocrine signals coordinate to achieve follicular development, oocyte maturity, and ovulation [4,5]. It seems that molecules with immune function constitute important components of the microenvironment regulating the physiological equilibrium in this process. Controlling inflammation in this context is vital for follicular rupture, tissue remodeling, and formation of corpus luteum. Nonetheless, abnormal control of inflammatory responses can negatively affect oocyte quality and embryo development [6,7].

Tumor necrosis factor- α (TNF- α) is a key proinflammatory cytokine that has been widely studied in the field of reproductive and endocrinology [8]. TNF- α is produced by several ovarian cells, like granulosa, theca cells as well as infiltrating immune cells, and plays important role in modulating follicular development [8–10]. Mechanistically, TNF- α mediates its physiological actions via activation of intracellular signal transduction pathways like NF- κ B and MAPK that control gene regulation responsible for processes such as apoptosis, inflammation, and proliferation [11,12]. By affecting those pathways, TNF- α regulates granulosa cell survival, steroid hormone synthesis, and follicular atresia. The effect of TNF- α on the ovary is two-faced and dose-dependent [13,14]. Under physiological conditions, TNF- α participates in follicular development and ovulation by inducing regulated apoptosis and tissue remodeling. In contrast, increased concentrations of TNF- α result in the induction of exaggerated inflammatory and oxidative responses, causing disturbances in mitochondrial function and steroid biosynthesis, as well as deterioration of oocyte quality. As a result, fertilization capacity and embryo viability may be compromised, indicating a possible mediator role of TNF- α in inflammation-mediated infertility [13].

In contrast, growth hormone (GH) is an important endocrine hormone which is associated with multiple functions related to growth, metabolism, and reproduction. GH is synthesized and secreted by the anterior pituitary and acts directly on cells as well as indirectly through the induction of insulin-like growth factor (IGF) [15]. In the ovary, GH increases follicular growth via proliferation of granulosa cells, improves follicular sensitivity to gonadotrophins, and promotes steroidogenesis. Mechanisms by which GH exerts its biological actions involve not only intracellular signaling pathways (such as JAK/STAT), but also cross-talks between other hormonal pathways (such as PI3K/Akt from IGF pathway) [16]. Another important effect of GH is improvement in oocyte competence due to cytoplasmic and nuclear maturation, increased activity of mitochondria, and enhancement of energy metabolism in oocytes. GH treatment was proposed as an auxiliary therapy for poor ovarian responders to achieve better results in oocyte collection and embryo development. The intrinsic role of GH as a marker of ovarian function and the association between the serum level of GH and outcomes of infertility treatments need further investigation [15,17].

However, emerging evidence shows that inflammation and endocrine signaling are not isolated from each other; on the contrary, there is significant interaction between these two pathways in regulating reproductive functions [18]. The actions of TNF- α and GH could be complementary, synergic, and even contrasting based on the concentration and timing of these factors in the follicular microenvironment. For instance, TNF- α -driven inflammatory pathways could affect the expression and action of GH receptors and vice versa. Ultimately, the complex interrelationship could be decisive regarding follicular growth, oocyte quality, and embryo competence [2,19]. In spite of evident biological significance of these interactions, research involving combined assessment of the role of TNF- α and GH in in vitro fertilization has been relatively limited, and most of the findings inconsistent. While some researchers have established a negative correlation between high levels of TNF- α and fertility, other studies indicated the necessity of moderate concentrations of this factor for normal ovarian physiology [18,19]. Moreover, while there is evidence suggesting that GH positively correlates with better ovarian response in certain groups of patients, the value of the biomarker for predicting pregnancy remains debatable [20].

From a clinical perspective, define a reliable biomarker may able to reflect the inflammation status, as well as endocrine function, will contribute to improving the analysis of ovarian reaction and increase individualization of patients' therapy during ICSI process. It is important to learn about the relationship between these cytokines and the development of egg cells and embryos as well as pregnancies' results. The role of these factors as independent markers of the effectiveness of ICSI procedures is still unknown [14,15,18,19]. For these reasons, the main objective of the current study was to determine the level of TNF- α and GH in blood and follicular fluid and to establish their relationship with the ovarian response, hormone concentration, oocytes and embryos qualities, as well as pregnancies' results in women who undergo assisted reproduction technology.

PATIENTS, MATERIALS AND METHODS

Design of Study and Subjects

The present prospective comparative study was conducted at Iraq's "High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University/Baghdad/Iraq" infertility center and was approved by Mansoura University for its validity. The approval of the local medical ethical committee of the IRB of Mansoura University, Egypt, was obtained, (approval code: MDP.24.10.167). The study was performed from January 2025 through January 2026. Informed written consent was taken from all subjects before inclusion in the study. One hundred and twenty infertile women who had been subjected to intracytoplasmic sperm injection (ICSI) were recruited into the study. Their age ranged from 20 to 40 years and were stratified into poor responders ($n = 40$), normal responders ($n = 40$), and hyper responders ($n = 40$, including patients with polycystic ovary syndrome).

Inclusion and exclusion criteria

Inclusion Criteria: Women undertaking ICSI treatment cycles, Poor, normal, and hyper responders, including women with PCOS, Men with normozoospermia status

Exclusion Criteria: History of any systemic disorders, Recent infections Patients on immunosuppressants, Cases of male-factor infertility such as, azoospermia and epididymal sperm extraction

Clinical hormonal analysis

All subjects received comprehensive medical evaluation based on medical history, age, infertility cause and duration, number of unsuccessful prior ICSI procedures, and body mass index (BMI) determined from the formula: BMI = Weight in kilograms/Height in meters squared. Hormonal levels were measured at baseline through the early follicular stage of the menstrual cycle, days one to two, and included: Anti-Müllerian hormone (AMH), Estradiol (E2), Luteinizing hormone (LH), Follicle stimulating hormone (FSH), Thyroid-stimulating hormone (TSH) Prolactin (PRL).

Controlled Ovarian Stimulation and ICSI Process

The protocol of a GnRH antagonist was used in controlled ovarian stimulation. The ovarian stimulation process initiated on the 2nd day of the menstrual cycle using recombinant FSH, dose being adjusted according to the individual patient characteristics. Ovulation induction was triggered with hCG when at least two follicles reached diameter 17–18 mm. Oocyte collection was done 34–36 hours after hCG administration under transvaginal ultrasound guidance. Following fertilization, embryos were cultured and examined morphologically and mature MII oocytes were selected for ICSI. Fertilization was proved

by two pronuclei appearance 2PN. Further monitoring of embryo development was conducted until cleavage stage Day3. Embryos were transferred at day 3 post fertilization depending on the state of their development. Pregnancy determination was done 14 days after embryo transfer

Collection of Samples

Three types of biological material were collected at three different stages: serum obtained on oocyte retrieval day (ORD); follicular fluid collected on oocyte retrieval day; serum obtained on embryo transfer day (ETD).

3-mL blood samples were centrifuged at 5000 rpm for 10 minutes, and the supernatant containing serum was isolated and kept in a fridge at -20°C before further analysis. Follicular fluid samples were centrifuged and kept under the same conditions.

Assessment of TNF- α and GH

TNF- α and GH were measured in serum and follicular fluid using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Briefly, the test based on standard ELISA principles. Optical density was measured at 450 nm using a microplate reader, and concentrations were calculated using standard calibration curves. All samples were processed under standardized laboratory conditions to ensure accuracy and reproducibility.

Characteristics of Oocytes and Embryos

Oocytes' morphology and maturity were estimated after the procedure of retrieval and denudation. Oocytes' maturity can be estimated by dividing them into four categories: Germinal Vesicle (GV), Metaphase I (MI), Metaphase II (MII) Abnormal oocytes, Fertilization was considered as proven by two pronuclei' presence (2PN) approximately 16–18 hours after intracytoplasmic sperm injection (ICSI). It allowed estimating fertilization rate and cleavage rate. The evaluation of the embryo's quality occurred in accordance with morphological criteria and classified into three grades: Grade 1 (high quality), Grade 2 (moderate quality), Grade 3 (low quality)

Statistical Analyses

SPSS software version 23.0 was used for the statistical analysis. For assessing the normal distribution of variables, Kolmogorov-Smirnov and Shapiro-Wilk tests were applied. As most variables were not normally distributed, median and interquartile range (IQR) were presented. The comparisons between groups were done using Kruskal-Wallis test. Spearman's rank correlation coefficient (r) was utilized for correlation analysis. Statistical significance was considered as $p < 0.05$.

RESULTS

TNF- α and GH Concentration Distributions

Distributions of TNF- α and GH concentrations in serum and follicular fluid samples were evaluated using Kolmogorov-Smirnov test and Shapiro-Wilk test. Most of the parameters showed a non-Gaussian distribution ($p < 0.05$), indicating the use of non-parametric statistics. Accordingly, inter-group analyses were conducted based on ovarian response groups.

Comparison of TNF- α and GH Across Ovarian Response Groups

Statistically significant differences between TNF- α and GH concentrations were found between poor, normal, and hyper-responder groups ($p < 0.05$, Table1). The concentrations of both biomarkers were found to be higher in the hyper responder group than in the normal and poor responders. These results imply that inflammatory and hormonal activities are associated with ovarian response. To determine if there are underlying mechanisms in endocrine control, hormonal profiles were also examined.

Table1: TNF- α and GH levels across ovarian response groups

Marker	Sample	Poor Responders	Normal Responders	Hyper Responders	p-value
TNF- α	Serum (ORD)	142.3 (55.2)	118.6 (49.7)	198.4 (62.1)	<0.001

	Follicular fluid	160.5 (48.3)	135.7 (51.2)	210.6 (70.5)	<0.001
	Serum (ETD)	150.2 (60.1)	130.8 (55.6)	185.3 (58.9)	<0.001
GH	Serum (ORD)	3.2 (1.4)	2.8 (1.2)	4.5 (1.6)	<0.001
	Follicular fluid	3.5 (1.5)	3.0 (1.3)	4.8 (1.7)	<0.001
	Serum (ETD)	3.0 (1.2)	2.6 (1.1)	4.1 (1.5)	<0.001
Values are presented as median (interquartile range). Comparisons between groups were performed using the Kruskal–Wallis test. ORD: oocyte retrieval day; ETD: embryo transfer day.					

Association of TNF- α and GH with Hormonal Profiles

Through correlation analysis, it was found out that TNF- α had significant correlations with various reproductive hormones, especially estrogen and LH, implying its effect on ovarian endocrine functioning, Table2. On the other hand, GH also exhibited a significant positive correlation with hormonal markers, reflecting its contribution to follicle sensitivity and hormone production. Both of these markers are thus involved in hormonal functioning, but through entirely separate mechanisms. The former through inflammation, while the latter through endocrine control. Because of the close link between hormonal functioning and follicular growth, it is logical to examine if these markers are affected by overall patient characteristics.

Table2: Correlation of TNF- α and GH with hormonal parameters

Marker	Sample	AMH (r, p)	E2 Trigger (r, p)	LH (r, p)
TNF-α	Serum (ORD)	0.295, 0.005	0.322, 0.002	0.301, 0.004
	Follicular fluid	0.410, <0.001	0.385, <0.001	0.352, 0.001
GH	Serum (ORD)	0.310, 0.003	0.338, 0.001	0.297, 0.005
	Follicular fluid	0.365, 0.001	0.402, <0.001	0.318, 0.002
Correlation analysis was performed using Spearman's rank correlation coefficient (r). AMH: anti-Müllerian hormone; E2: estradiol; LH: luteinizing hormone.				

Association with General Characteristics

However, both TNF- α and GH had only minimal relationships with clinical variables. Although there was a mild correlation with age, none of the markers had noticeable correlation with BMI, time of infertility, number of ICSI procedures, or nature of infertility. TNF- α showed a weak but statistically significant negative correlation with age ($r = -0.210$, $p = 0.045$), while GH demonstrated a non-significant trend ($r = -0.185$, $p = 0.072$). No significant associations were observed between TNF- α or GH and BMI (TNF- α : $r = 0.102$, $p = 0.321$; GH: $r = 0.090$, $p = 0.381$), duration of infertility (TNF- α : $r = -0.080$, $p = 0.442$; GH: $r = -0.050$, $p = 0.612$), or number of ICSI trials (TNF- α : $r = -0.060$, $p = 0.558$; GH: $r = -0.040$, $p = 0.701$), Table3. This indicates that TNF- α and GH are indicators of biological events occurring in the ovary rather than being influenced by demographic variables. Since these markers are related to ovarian activity and hormone levels, we then examined their relationship with oocyte quality.

Table3: Correlation with general characteristics

Marker	Sample	Age (r, p)	BMI (r, p)	Duration (r, p)	ICSI Trials (r, p)
TNF-α	Serum (ORD)	-0.210, 0.045	0.102, 0.321	-0.080, 0.442	-0.060, 0.558
GH	Serum (ORD)	-0.185, 0.072	0.090, 0.381	-0.050, 0.612	-0.040, 0.701
Spearman's correlation coefficient (r) was used. BMI: body mass index; ICSI: in vitro fertilization.					

Association of TNF- α and GH with Oocyte Characteristics

In view of the previously mentioned correlations of TNF- α and GH with ovarian response and hormonal activity, we sought to explore their associations with oocytes' features. The data in Table4 refers that Both TNF- α and GH displayed significant associations with oocytes' characteristics, specifically the total number of oocytes retrieved, the number of oocytes at the germinal vesicle (GV) stage, and the number of mature oocytes at the metaphase II (MII) stage. Regarding TNF- α , its concentration in the serum on the oocyte retrieval day (ORD) correlated positively and significantly with the total number of oocytes ($r = 0.352$, $p = 0.001$), GV oocytes ($r = 0.310$, $p = 0.003$), and MII oocytes ($r = 0.285$, $p = 0.007$). More pronounced associations were found for the concentration of TNF- α in follicular fluid, being positively correlated with the total number of oocytes ($r = 0.480$, $p < 0.001$), GV oocytes ($r = 0.401$, $p < 0.001$), and MII oocytes ($r = 0.365$, $p = 0.001$). In turn, GH concentration in the serum on the ORD showed significant positive correlations with the total number of oocytes ($r = 0.330$, $p = 0.002$), GV oocytes ($r = 0.290$, $p = 0.005$), and MII oocytes ($r = 0.260$, $p = 0.011$). In opposition to its systemic effects, the concentrations of GH in follicular fluid were significantly negatively associated with MII oocytes ($r = -0.210$, $p = 0.042$). Consequently, TNF- α and GH appear to be significantly correlated with the number of oocytes and their maturation status. While TNF- α seems to show a consistent positive effect, especially concerning the local follicular microenvironment, GH displays a variable effect depending on the compartment. Thus, based on the findings presented, we then investigated the associations of TNF- α and GH with embryo development and quality.

Table4: Correlation with oocyte characteristics

Marker	Sample	Total (r, p)	GV (r, p)	MI (r, p)
TNF-α	Serum (ORD)	0.352, 0.001	0.310, 0.003	0.285, 0.007
	Follicular fluid	0.480, <0.001	0.401, <0.001	0.365, 0.001
GH	Serum (ORD)	0.330, 0.002	0.290, 0.005	0.260, 0.011
	Follicular fluid	0.295, 0.004	0.270, 0.008	-0.210, 0.042
Spearman's correlation coefficient (r) was used. GV: germinal vesicle; MI: metaphase II oocytes.				

Correlations between TNF- α and GH with Embryo Characteristics

Having analyzed the associations of the levels of TNF- α and GH with ovarian response and oocyte characteristics, we examined the correlation of the factors with embryo development and quality. Generally, both TNF- α and GH were found to be

associated with embryo characteristics; nevertheless, the correlations with these factors proved to be less stable than in the case of oocyte characteristics, Table5.

As regards TNF- α , its serum level on oocyte retrieval day was positively correlated with grade 1 embryos ($r = 0.220$, $p = 0.034$). No significant correlation was found between TNF- α serum level and grade 2 embryos ($r = -0.150$, $p = 0.140$) or grade 3 embryos ($r = -0.180$, $p = 0.082$). The above correlations suggest that TNF- α exerts an effect on certain embryo characteristics but not all of them. As for GH, no significant correlations were found between GH and grade 1 embryos ($r = 0.190$, $p = 0.067$) or lower-grade embryos. These results show that GH affects the development of embryos weakly or even insignificantly. Thus, TNF- α might have an effect on embryo quality promoting the formation of embryos of high quality. However, GH is unlikely to exert any effect on embryo development or quality. Given that embryo quality determines the success rate of ICSI, we decided to investigate the possible associations between TNF- α and GH and pregnancy outcomes.

Table5: Correlation with embryo characteristics

Marker	Sample	Grade 1 (r, p)	Grade 2 (r, p)	Grade 3 (r, p)
TNF-α	Serum (ORD)	0.220, 0.034	-0.150, 0.140	-0.180, 0.082
GH	Serum (ORD)	0.190, 0.067	-0.120, 0.248	-0.100, 0.320
Spearman's correlation coefficient (r) was used. Embryo grading was based on standard morphological criteria (Grade 1: high quality; Grade 2: moderate; Grade 3: low quality).				

Analysis of Pregnancy Outcome with Prediction

Despite the strong correlations of TNF- α and GH with ovarian responsiveness, hormonal profile, and oocyte features, none of these markers could be used as statistically significantly differentiating markers of pregnancy and negative pregnancy outcomes. TNF- α levels on ETD in the pregnancy group were slightly but insignificantly lower than those in the non-pregnant group (165.4 [55.2] vs. 185.3 [58.9]; $p = 0.082$). The same observation could be made for the GH levels, which did not differ significantly between the groups either (3.2 [1.3] vs. 3.5 [1.4]; $p = 0.215$). The predictive performance of both TNF- α and GH was assessed using ROC analysis. Thus, TNF- α at ETD had moderate predictability; the AUC of 0.778 suggested that TNF- α was capable of discriminating between the groups with a cutoff value ≤ 185.32 that resulted in a sensitivity and specificity of 84.6% and 64.7% respectively. As for GH, its low predictive performance is proven by the low AUC (0.58), which makes any cut-off values insignificant from the clinical perspective, Table6 and Table7. Collectively, it may be concluded that TNF- α and GH have predictive power, but only to a limited extent.

Table6: Comparison with pregnancy outcome

Marker	Pregnancy (+)	Pregnancy (-)	p-value
TNF- α (ETD)	165.4 (55.2)	185.3 (58.9)	0.082
GH (ETD)	3.2 (1.3)	3.5 (1.4)	0.215
Values are presented as median (interquartile range). Comparisons were performed using the Mann-Whitney U test. ETD: embryo transfer day.			

Table7: ROC analysis

Marker	Cutoff	AUC	Sensitivity	Specificity
TNF- α (ETD)	≤ 185.32	0.778	84.60%	64.70%
GH	—	0.58	—	—
ROC: receiver operating characteristic; AUC: area under the curve. Sensitivity and specificity were calculated at the optimal cutoff value.				

DISCUSSION

The present study provides comprehensive evidence that both tumor necrosis factor- α (TNF- α) and growth hormone (GH) are significantly associated with ovarian response, hormonal activity, and oocyte characteristics in women undergoing ICSI. These findings highlight the important roles of inflammatory and endocrine pathways in regulating the follicular microenvironment and supporting early stages of reproductive function.

One of the main findings of this study is the strong significant elevated of TNF- α and GH levels in hyper responder patients compared to the other suggesting that endocrine activity and inflammatory cascade are closely linked to ovarian responsiveness [21,22]. Previously, studies indicated that regulated inflammatory process are crucial for normal follicular development, tissue remodeling and ovulation. For instance, TNF- α has been shown to regulate granulosa cell function and steroidogenesis through activation of intracellular pathways such as NF- κ B and MAPK [23,24]. At physiological levels, this cytokine contributes to follicular maturation; however, excessive expression may lead to oxidative stress and impaired cellular function. More likely, increased levels of GH found in hyper responders support it's a well-established role in enhancing follicular sensitivity to gonadotropins and promoting steroidogenesis. GH exerts its effects through activation of the JAK/STAT signaling pathway and through stimulation of insulin-like growth factor (IGF) production, which amplifies follicular growth and oocyte maturation. These mechanisms collectively explain the observed association between GH levels and ovarian response [17,25].

The strong correlations between TNF- α and GH and the important reproductive hormones such as AMH, estradiol, and LH further emphasize the importance of these molecules in hormone-mediated communication. AMH is an indicator of the ovarian reserve, while estradiol and LH are markers for follicular health and ovulation capacity. Therefore, the relationships found here suggest that TNF- α and GH can act as mediators integrating hormonal signaling and follicular activities. This is consistent with previous studies showing that inflammatory cytokines and growth factors cooperatively participate in the regulation of ovarian physiology [26,27].

Regarding oocyte features, both TNF- α and GH significantly positively correlated with the total number of oocytes and oocyte maturation levels, especially from a systemic perspective. It was implied that a well-regulated inflammation status and hormonal signaling were essential in the follicular development process and the oocyte quality. Notably, the correlations between TNF- α and oocyte parameters were significantly higher in the follicular fluid [19,28,29]. This suggests a stronger local effect of this molecule, implying that cytokine-mediated signal transduction within the follicle is critical for oocyte development. On the other hand, GH showed a more complex pattern, with systematic positive correlations but had a negative association with mature eggs in follicular fluid. It is apparent that the local control of GH within the follicle is different from its systemic effects, possibly due to feedback controls or differential sensitivity of its receptors. However, the findings of embryo features revealed weaker and less consistent correlation compared to oocyte-related results [2,30]. TNF- α was observed to have an insignificant yet positive correlation with grade 1 embryos; this could imply that some controlled inflammation might aid in early embryogenesis. Yet, these correlations were not observed between TNF- α and all grades of embryos. Likewise, there were weak and insignificant correlations seen between GH and the grade of embryos; the role of GH could thus be limited to follicular phases only [31]. The lack of the significant association between TNF- α and GH levels and clinical pregnancy outcomes was the key finding of this study. Although both markers showed a strong correlation with ovarian function and egg quality, none showed a statistical difference between the pregnant and non-pregnant groups. This finding emphasizes the clear distinction

between biomarkers for ovarian function and biomarkers for embryo implantation success [32,33]. Notably, TNF- α at embryo transfer day had moderate prognostic value as it yielded an AUC score of 0.778 and was sensitive; however, its specificity was still moderately low and no statistically significant group differences could be seen. The poor prediction value of GH in this research confirmed the previously stated hypothesis of GH's low clinical significance. Indeed, the research findings align with those of earlier publications which indicated that biomarkers showing follicular growth cannot serve as effective predictors of pregnancy [34]. There are numerous factors involved in implantation and fertilization success, such as endometrium receptivity, embryo genetic competence, as well as immune factors present in maternal-fetal interactions, all of which have been shown to significantly impact pregnancy outcomes [35]. Thus, while TNF- α and GH could provide insights into ovarian and follicular health, they would fail to consider all aspects of embryo implantation. Thus, from a practical point of view, the current research shows that TNF- α and GH can be considered as biomarkers of ovarian and follicular activity, but cannot be used as effective predictors on their own in order to forecast ICSI success. While TNF- α and GH could be helpful in evaluating the state of the ovaries and could be taken into account in the course of stimulation protocol selection, it should be done in combination with other markers [36].

Thus, TNF- α and GH, as inflammatory and endocrine factors, respectively, show how complex the field of reproductive biology actually is since these hormones seem to play critical parts in reproductive success, as shown by the study. Specifically, while these markers are active in follicular development and ovulation stages, they become irrelevant later, such as when implantation happens. Further research in the field should take into account all three factors mentioned in the study to find out whether there is any correlation between all three and whether it could predict ICSI outcome.

CONCLUSION

In conclusion, this study revealed that TNF- α and GH have an influence on ovarian responsiveness and hormonal status as well as the features of oocytes in women who undergo ICSI procedure. The both biomarkers have higher concentrations among hyper responders and are significantly correlated with reproductive hormones and oocyte parameters, which indicates the possible regulatory roles played by these cytokines in the inflammatory and endocrine environment in the follicle. TNF- α demonstrated much stronger associations as compared to GH and had more pronounced local influence on ovarian response and oocyte quality, whereas the role of growth hormone is more complicated and variable. Nonetheless, neither TNF- α , nor GH have any statistically significant differences between pregnant and non-pregnant women. Despite some correlations with pregnancy in TNF- α group, neither of the two biomarkers is a good independent predictor of ICSI success based on ROC analysis results. Thus, it seems that TNF- α and GH are indicators of ovarian and follicular functioning, not of successful implantation. However, there are many other factors that determine ICSI outcomes. Therefore, in order to better predict ICSI success, more comprehensive studies involving all aspects should be conducted.

Recommendations

On the basis of the results from the present investigation, TNF- α and GH can prove to be valuable auxiliary indicators for determining the response and follicular function in ICSI candidates; nevertheless, they cannot be relied upon exclusively for predicting pregnancy outcomes. It is highly advisable that future researchers should attempt to include inflammatory and follicular biomarkers along with hormonal indicators in their predictions. The specific contributions of systemic and follicular concentrations of these biomarkers need to be delineated, and their mechanisms also deserve to be investigated further.

REFERENCES

- [1] Barroso-Villa G, Valdespin-Fierro C, Weiser-Smeke AE, Machargo-Gordillo AP, Flores-Pliego A, Palma-Lara I, et al. Follicular fluid biomarkers for prediction of human ICSI outcome in women with poor ovarian response. *Middle East Fertil Soc J*. 2023;28(1). doi:10.1186/s43043-023-00128-8
- [2] Molka B, Gwladys B, Dorian B, Lucie M, Mustapha B, Rosalie C, et al. Follicular Fluid Growth Factors and Interleukin Profiling as Potential Predictors of ICSI Outcomes. *Front Physiol*. 2022;13. doi:10.3389/fphys.2022.859790
- [3] Albeitawi S, Bani-Mousa SU, Jarrar B, Aloqaily I, Al-Shloul N, Alsheyab G, et al. Associations Between Follicular Fluid Biomarkers and ICSI/ICSI Outcomes in Normo-Ovulatory Women—A Systematic Review. *Biomolecules*. 2025. doi:10.3390/biom15030443

- [4] Simon LE, Rajendra Kumar T, Duncan FE. In vitro ovarian follicle growth: A comprehensive analysis of key protocol variables. *Biology of Reproduction*. 2020. doi:10.1093/biolre/ioaa073
- [5] Hennet ML, Combelles CMH. The antral follicle: A microenvironment for oocyte differentiation. *International Journal of Developmental Biology*. 2012;56(10–12). doi:10.1387/ijdb.120133cc
- [6] Karimi S, Baharaghdam S, Danaei S, Yousefi M. Embryo-maternal cross-talk: key players in successful implantation and live birth rates. *Reproductive Biology and Endocrinology*. 2025. doi:10.1186/s12958-025-01477-x
- [7] Fujiwara H, Ono M, Sato Y, Imakawa K, Iizuka T, Kagami K, et al. Promoting roles of embryonic signals in embryo implantation and placentation in cooperation with endocrine and immune systems. *International Journal of Molecular Sciences*. 2020. doi:10.3390/ijms21051885
- [8] Terranova PF, Montgomery Rice V. Review: Cytokine involvement in ovarian processes. *American Journal of Reproductive Immunology*. 1997. doi:10.1111/j.1600-0897.1997.tb00192.x
- [9] Lliberos C, Liew SH, Zareie P, La Gruta NL, Mansell A, Hutt K. Evaluation of inflammation and follicle depletion during ovarian ageing in mice. *Sci Rep*. 2021;11(1). doi:10.1038/s41598-020-79488-4
- [10] Terranova PF. Potential roles of tumor necrosis factor- α in follicular development, ovulation, and the life span of the corpus luteum. *Domestic Animal Endocrinology*. 1997. doi:10.1016/S0739-7240(96)00094-X
- [11] Xue H, Hu Z, Liu S, Zhang S, Yang W, Li J, et al. The mechanism of NF- κ B-TERT feedback regulation of granulosa cell apoptosis in PCOS rats. *PLoS One*. 2024;19(10 October). doi:10.1371/journal.pone.0312115
- [12] Regan SLP, Knight PG, Yovich JL, Leung Y, Arfuso F, Dharmarajan A. Granulosa cell apoptosis in the ovarian follicle-A changing view. *Frontiers in Endocrinology*. 2018. doi:10.3389/fendo.2018.00061
- [13] Silva JRV, Lima FEO, Souza ALP, Silva AWB. Interleukin-1 β and TNF- α systems in ovarian follicles and their roles during follicular development, oocyte maturation and ovulation. *Zygote*. 2020. doi:10.1017/S0967199420000222
- [14] Liang H, Xu D, Tong X, Ling B, Wang W, Feng D, et al. Biphasic Effect of TNF- α on the Survival and Endocrine Function of Human Ovarian Granulosa Cells. *Clin Exp Obstet Gynecol*. 2022;49(5). doi:10.31083/j.ceog4905119
- [15] Nishad R, Mukhi D, Menon RK, Pasupulati AK. Growth Hormone and Metabolic Homeostasis. *EMJ Diabetes*. 2018. doi:10.33590/emjdiabet/10313977
- [16] Ipsa E, Cruzat VF, Kagize JN, Yovich JL, Keane KN. Growth Hormone and Insulin-Like Growth Factor Action in Reproductive Tissues. *Frontiers in Endocrinology*. 2019. doi:10.3389/fendo.2019.00777
- [17] Chang CW, Sung YW, Hsueh YW, Chen YY, Ho M, Hsu HC, et al. Growth hormone in fertility and infertility: Mechanisms of action and clinical applications. *Frontiers in Endocrinology*. 2022. doi:10.3389/fendo.2022.1040503
- [18] Shirafuji A, Orisaka M, Shimizu-Mizuno N, Uesaka M, Fujita Y, Mizuno K, et al. Pro-inflammatory cytokines disrupt in vitro preantral follicle development by targeting granulosa and theca cell functions. *Front Endocrinol (Lausanne)*. 2026;16. doi:10.3389/fendo.2025.1667019
- [19] Huang Y, Cheng Y, Zhang M, Xia Y, Chen X, Xian Y, et al. Oxidative stress and inflammatory markers in ovarian follicular fluid of women with diminished ovarian reserve during in vitro fertilization. *J Ovarian Res*. 2023;16(1). doi:10.1186/s13048-023-01293-0
- [20] Pan Y, Pan C, Zhang C. Unraveling the complexity of follicular fluid: insights into its composition, function, and clinical implications. *Journal of Ovarian Research*. 2024. doi:10.1186/s13048-024-01551-9
- [21] Piccinni MP, Vicenti R, Logiodice F, Fabbri R, Kullolli O, Pallecchi M, et al. Description of the Follicular Fluid Cytokine and Hormone Profiles in Human Physiological Natural Cycles. *Journal of Clinical Endocrinology and Metabolism*. 2021;106(2). doi:10.1210/clinem/dgaa880
- [22] Wang C, Feng Y, Chen Y, Lin X, Li X. Proximity extension assay revealed novel inflammatory biomarkers for follicular development and ovarian function: a prospective controlled study combining serum and follicular fluid. *Front Endocrinol (Lausanne)*. 2025;16. doi:10.3389/fendo.2025.1525392
- [23] Li X, Li C, Lee X, Yang J, Lin M, Zhou X, et al. ASSOCIATION OF LEVELS OF ADIPOKINES AND CYTOKINES IN INDIVIDUAL FOLLICLES WITH IN VITRO FERTILIZATION OUTCOMES IN WOMEN WITH DIFFERENT OVARIAN RESERVE. *Fertil Steril*. 2024;122(4). doi:10.1016/j.fertnstert.2024.07.771
- [24] Li X, Li C, Yang J, Lin M, Zhou X, Su Z, et al. Associations of the levels of adipokines and cytokines in individual follicles with in vitro fertilization outcomes in women with different ovarian reserves. *Journal of Ovarian Research*. 2025;18(1). doi:10.1186/s13048-025-01594-6