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A New Trend of Ovarian and Endometrial Biomarkers in Predicting Assisted Reproductive Technology (ART) Outcomes

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Abstract

Background: Various models have been developed for the prediction of pregnancy after in vitro fertilization (IVF). These models differ from one another in the predictors they include. We performed a systematic review and meta-analysis to identify the most relevant predictors for success in IVF.

Objectives: The aim of the current study was to determine whether endometrial receptivity biomarkers Mucin1(MUC1), Growth Differentiation Factor -9 (GDF-9), and Human Leucocyte Antigen (HLA) are associated with IVF outcomes.

Materials and Methods: In order to achieve this objective, 120 females with primary or secondary infertility undergoing In Vivo Fertilization (IVF) were included in this study. Serum levels of mucin1(MUC1), Growth Differentiation Factor -9 (GDF-9), and Human Leucocyte Antigen (HLA) were measured; types of infertility and IVF outcomes (positive β-hCG) were recorded for each participant.

Results: Result were shown that females with secondary infertility have a significant higher mean level of both mucin (9.26) and GDF9 (2.7) compared to those with primary infertility (5.94 and 1.03, respectively). While Females with primary infertility have a highly significant higher mean HLA level (33.14) compared to those with secondary infertility (27.34). Also, the mean Mucin level was significantly higher in the group with a positive Beta hCG test (7.59 ng/ml) compared to the negative group (6.54 ng/ml), p-value was <0.05. Similar to Mucin, the mean GDF9 level is also higher in the positive Beta hCG group (3.62 ng/ml) compared to the negative group (2.22 ng/ml) p value was <0.001, increasing level of GDF9 might reflect successful implantation or early pregnancy. The mean HLA level was lower in the positive Beta hCG group (18.31) compared to the negative group (27.19), p value was <0.001.

Conclusion: biomarkers of endometrial receptivity, exhibit potential role as indicators of the ovarian response to stimulation during in vitro fertilization.

اتجاه جديد للمؤشرات الحيوية للمبيض وبطانة الرحم في التنبؤ بنتائج التلقيح الاصطناعي

زينب أحمد الجراح , رنا مجيد حميد, وسن غازي الصافي

الملخص

الخلفية: تم تطوير نماذج مختلفة للتنبؤ بالحمل بعد التخصيب في المختبر (.(IVFوتختلف هذه التطورات عن بعضها البعض في التوقعات التي تحتويها. تم إجراء مراجعة للدراسة لتحليل أهم العوامل التي تنبئ بنجاح التلقيح الصناعي.

الأهداف: كان الهدف من الدراسة الحالية هو تحديد ما إذا كانت المؤشرات الحيوية لتقبل بطانة الرحم (MUC1) Mucin1، وعامل تمايز النمو -9 ((GDF-9، ومستضد الكريات البيض البشرية ((HLAمرتبطة بنتائج التلقيح الاصطناعي.

المواد والطرق: من أجل تحقيق هذا الهدف، تم تضمين 120 أنثى تعاني من العقم الأولي أو الثانوي وتخضع للتخصيب في الجسم الحي ((IVFفي هذه الدراسة. تم قياس مستويات مصل (MUC1) mucin1، وعامل تمايز النمو -9 ((GDF-9، ومستضد الكريات البيض البشرية ((HLA؛ تم تسجيل أنواع العقم ونتائج التلقيح الصناعي (β-hCG الإيجابية) لكل مشارك

النتائج: أظهرت النتائج أن الإناث المصابات بالعقم الثانوي لديهن مستوى متوسط أعلى بكثير لكل من الميوسين (9.26) و (2.7) GDF9مقارنة مع أولئك الذين يعانون من العقم الأولي (5.94 و 1.03 على التوالي). في حين أن الإناث المصابات بالعقم الأولي لديهن متوسط أعلى بكثير لمستوى (33.14) HLAمقارنة بالنساء المصابات بالعقم الثانوي (27.34). أيضًا، كان متوسط مستوى الميوسين أعلى بكثير في المجموعة ذات اختبار Beta hCG الإيجابي (7.59 نانوغرام/مل) مقارنة بالمجموعة السلبية (6.54 نانوغرام/مل)، وكانت القيمة .0.05> pعلى غرار Mucin، يكون متوسط مستوى GDF9 أعلى أيضًا في مجموعة Beta hCG الإيجابية (3.62 نانوغرام/مل) مقارنة بالمجموعة السلبية (2.22 نانوغرام/مل) وكانت قيمة 0.001> p، وقد يعكس مستوى GDF9 المتزايد نجاح عملية الزرع أو الحمل المبكر. الحمل. كان متوسط مستوى HLA أقل في مجموعة Beta hCG الإيجابية (18.31) مقارنة بالمجموعة السلبية (27.19)، وكانت قيمة .0.001> p

الاستنتاج: المؤشرات الحيوية لتقبل بطانة الرحم لها دور محتمل كمؤشرات لاستجابة المبيض للتحفيز أثناء الإخصاب في المختبر.

1. Introduction

Assisted Reproductive Technology (ART) is a group of medical procedures for treating the infertile human in which both male and female gametes are used outside the body (in vitro) to attain pregnancy. It includes, in vitro fertilization IVF and other techniques (Heber et al., 2022).Assisted Reproductive Technique has been used increasingly over the years as an infertility treatment globally between women of different ages since the first baby Louise Brown was born as a result of in vitro fertilization in the UK in 1978 (Lepore and Petruzziello, 2021).Biomarkers serve as quantifiable biological indicators, signalling various medical conditions or states within an organism. These molecular beacons are instrumental for the early detection of a dynamic physiologic state or disease, tracking the efficacy of treatment and potentially predicting medical outcomes. They are part of a larger endeavor of precision or personalized medicine, which strives to optimize diagnostic testing and therapy for each patient. In assisted reproductive technologies (ART), the discovery of dependable biomarkers related to ovarian and endometrial function is pivotal in elevating ART procedure successes, with the aspiration of ultimately leading to the birth of a healthy child. Predictions of ovarian response and endometrial receptivity are informative to setting realistic patient and physician expectations, tailoring treatment approaches, reducing complications, and elevating the success rates of ART treatment (Volovsky and Seifer, 2024). The knowledge gap on this topic might be the need to explore the existing scope and examining ovarian and endometrial biomarkers in their respective roles in ART outcomes. this study would include both traditional and possible emerging biomarkers with a discussion of both serum markers. serum ovarian biomarkers provide readily accessible methods through venipuncture, it may ultimately reveal future non-invasive ovarian biomarkers that are currently not realized (Volovsky and Seifer, 2024). In tandem with ovarian function, the endometrial environment is vital in completing the fertility puzzle. The endometrium's receptivity, a critical determinant for successful implantation, is being increasingly deciphered by various biomarkers. Together, ovarian and endometrial biomarkers have the potential to refine fertility treatments, ushering in an era of targeted therapy and improved prognostic capabilities in reproductive medicine. Therefore, this study aimed to examine the role of the proposed markers by estimating the level of MUC1, GDF-9 and HLA among inertial females undergoing IVF.

2. Materials and Method

2.1.Study Participants

This study was conducted at the Fertility Center of Al-Kafeel super-specialty Hospital in Karbala, Iraq. A total of 120 women with primary or secondary infertility who were undergoing IVF treatment were recruited between December 2023 and July 2024. Women aged 21-42 years, diagnosed with primary or secondary infertility, undergoing IVF treatment with FSH stimulation, and having had serum AMH levels evaluated before treatment were included. The excluded criteria were females with endocrine disorders thyroid diseases and hypogonadotropic hypogonadism. Severe endometriosis. Previous ovarian surgery, radiation or chemotherapy and premature ovarian failure.

2.2.Biochemical Analysis

venous blood was withdrawn from all subjects for hormones analysis which was done at the 2nd day of menstrual cycle. The blood centrifuged for 15 minutes at a speed of 1000 xg and serum samples were placed into Eppendorf Tubes 1.5 mL stored in - 20℃ to be used for hormones analysis which was achieved using MiniVidas analysis equipment. An enzyme immunoassay technique (quantitative, sandwich) was used. MUC1, GDF-9 and HLA, specific

micro-plate which was pre-coated with a monoclonal antibody, Kits were purchased from Elabscience/ USA. Micro-plates wells were filled with standards and samples.

2.3.Statistical Analysis

The data analysis for this work was generated using the graphical Pad Prism. Descriptive statistics were performed on the data of each group. Values were illustrated by n (%) for categorical, Scale variables were presented by mean ± 2 standard deviation for normal data. Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p- values <0.05 (two-sided) were considered to be statistically significant.

3. Results

The demographics characteristic of the infertile female participants was shown the majority of participants fell within the 28–34-year age range. For primary infertility, more female factor (13.2%) and unexplained infertility (11.3%) were reported compared to secondary infertility. While Secondary infertility was a higher prevalence of male factors (42.9%) and combined infertility (21.4%). Most participants (around 87%) had undergone only one IVF attempt in both primary and secondary infertility groups.

3.1.Mean Differences of Biomarkers Based on The Types of Infertility

Table 1 shows the mean levels and standard deviations (SD) of three biomarkers, mucin, GDF9, and HLA, in infertile females categorized by primary and secondary infertility. Results were showed that females with secondary infertility have a significant higher mean level of both mucin (9.26) and GDF9 (2.7) compared to those with primary infertility (5.94 and 1.03, respectively). While Females with primary infertility have a highly significant a higher mean HLA level (33.14) compared to those with secondary infertility (27.34), as presented in Fig.1. Results were indicated the potential differences in biomarker levels between women with primary and secondary infertility. The increasing in mucin and GDF9 levels in secondary infertility might be related to underlying causes. The higher HLA levels in primary infertility could be indicative of an autoimmune response affecting fertility.

Table 1: Mean Differences of Biomarkers Levels: Mucin, GDF9 And HLA Among Group of Infertile Females Based on The Types of Infertility

	Mucin	**GDF9**	**HLA**
Primary Infertility	5.94±1.68	1.03±0.9	33.14±22.33
Secondary Infertility	9.26±3.28	2.7±2.3	27.34±13.5

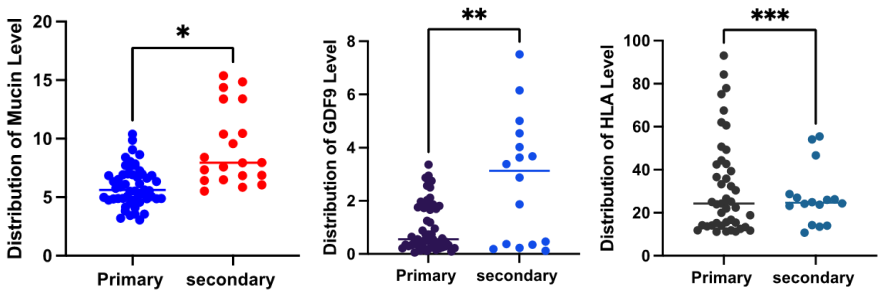

Figure 1: Distribution of Biomarkers Levels: Mucin, GDF9 and HLA among group of infertile females based on the types of infertility.

3.2.Mean Differences of Biomarkers Based on Final Beta Hcg Test

Table 2 illustrates the mean levels (with standard deviation) of Mucin, GDF9, and HLA in infertile females, categorized by the outcome of the final Beta hCG test (positive or negative). The mean Mucin level was significantly higher in the group with a positive Beta hCG test (7.59 ng/ml) compared to the negative group (6.54 ng/ml), p-value was <0.05. it might be due to their role in clinical utility in early pregnancy detection. Similar to Mucin, the mean GDF9 level is also higher in the positive Beta hCG group (3.62 ng/ml) compared to the negative group (2.22 ng/ml) p-value was <0.001, increasing level of GDF9 might reflect successful implantation or early pregnancy. The mean HLA level is lower in the positive Beta hCG group (18.31) compared to the negative group (27.19), p-value was <0.001, as presented in Fig.2. This finding might reflect a possible link between HLA expression and pregnancy success. Lower HLA levels might indicate a more favorable immune environment for implantation.

Table 2: Mean differences of Biomarkers Levels: Mucin, GDF9 and HLA among the group of infertile females based on the final Beta hCG test

	- ***Beta hCG***	+ ***Beta hCG***
Mucin	6.54±2.10	7.59±1.44
GDF9	2.22±2.13	3.62±2.95
HLA	27.19±14.75	18.31±6.26

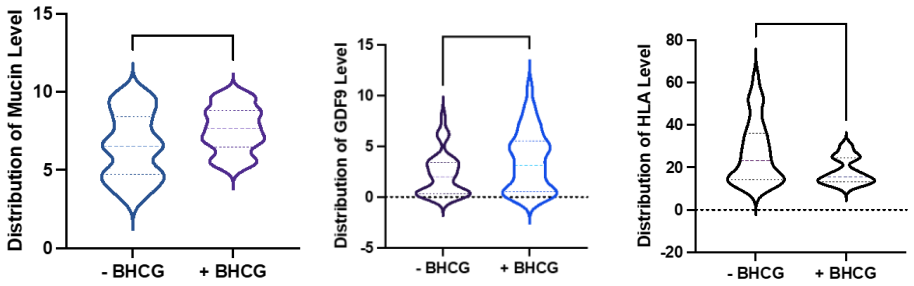

Figure 2: Distribution of Biomarkers Levels: Mucin, GDF9 and HLA among the group of infertile females based on the final Beta hCG test

4. Discussion

This study presented an interesting difference in the levels of three biomarkers, Mucin, GDF9, and HLA, between women with primary and secondary infertility. Women with secondary infertility had significantly higher mean levels of both Mucin and GDF9 compared to those with primary infertility. Elevated Mucin and GDF9 levels in secondary infertility could be a compensatory response to declining ovarian reserve or altered follicular dynamics (Demini et al., 2023) **.** On the other hand, Women with primary infertility had a significantly higher mean HLA level compared to those with secondary infertility. HLA molecules are involved in the immune system's response. Lower HLA levels in secondary infertility could indicate a less tolerant immune environment within the ovaries. This might be detrimental to implantation success. Previous pregnancies in women with secondary infertility might have modulated their immune response, leading to lower HLA expression. Alternatively, underlying causes of secondary infertility could be linked to altered immune regulation (Robertson et al., 2022). The current study also illustrated that Both Mucin and GDF9 levels showed a progressive increase with increasing AMH levels. increase in Mucin and GDF9 levels could be associated with a greater number of follicles or enhanced follicular activity in women with higher AMH, which is a marker of remaining ovarian reserve (Bilibio et al., 2020). Interestingly, HLA levels displayed an opposite trend. The highest levels were found in the subgroup lowest AMH and progressively decreased with increasing AMH. No Previous studies reported such findings, but many studies confirmed that the Anti-Mullerian hormone was highly related and predicting ovarian response to gonadotropin stimulation due to their role in ovarian primordial follicle recruitment and dominant follicle selection (Vijay et al., 2022). In line with the study of Hazout et al., greater AMH concentrations were associated with a higher number of mature oocytes, a higher number of embryos, followed by a greater clinical pregnancy (Hazout et al., 2004)**.** No previous studies reported such finding but A close explanation might be reflected by other research link the studied biomarkers and Endometrial receptivity.

Endometrial super fertility is clinically associated with inappropriate uterine receptivity, as it allows defective blastocysts to implant and start pregnancies that cannot be maintained with good health (Teklenburg et al., 2010). The glycoprotein MUC-1 is an anti-adhesion molecule that may play a role in endometrial super fertility. Mucin is also a major component of the apical surface of uterine epithelial cells, which are involved in the initial stages of embryo–uterine interactions during implantation. While uterine epithelial cells serve a protective function for the tissue, these cells must also allow the embryo to attach (Nordenmark et al., 2023). MUC1 (Mucin-1), also known as epithelial mucin, was the first mucin core protein to be cloned from both humans and mice. In a previous study, we observed numerous glycoprotein particles at the apex and base of endometrial epithelial cells (Suri et al., 2021). Expression of MUC-1 decreases to relatively low levels in healthy women during the implantation (window) to allow the possibility of contact between an embryo and the endometrium. On the other hand, previous studies suggest that MUC-1 expression is extremely low in the endometrium, during the implantation window, in women with recurrent pregnancy loss, compared with fertile women (Xu et al., 2012). The low levels of mucin-1 (MUC-1) may explain why patients seem exceptionally fertile despite having RIF and recurrent pregnancy loss. The significant differences in the mean serum concentrations of GDF9 among infertile females were in line with previous studies.

GDF9 is secreted by the oocyte and regulates the quality of the egg by interacting with the surrounding somatic cells. It is thus expected that GDF9 concentrations in human serum will vary with differing reproductive pathologies, and

recently we demonstrated evidence of an association between serum GDF9, and the number of oocytes retrieved during IVF (Shamsa et al., 2023). Mutations that reduce or disrupt the production of these oocyte-secreted factors have been observed in conditions associated with subfertility including polycystic ovary syndrome (PCOS), primary ovarian insufficiency (POI) and Turners syndrome (Patiño et al., 2017). An indication that aberrant GDF9 function may be associated with endometriosis arises from reports of lower concentrations of GDF9 protein and mRNA in follicular fluid of patients with severe endometriosis (Kawabe et al., 2015)**.** Regarding HLA, the current study was also consistence with the previous research who reported increased levels of HLA among infertile females.

During pregnancy, the maternal immune system is in close contact with the cells of the semiallogenic fetus, and a normal immunological homeostasis is needed to avoid rejection of the fetus (Monti et al., 2019). The human leukocyte antigen-G (HLA-G) molecules belong to the nonclassical HLA class Ib family and have immunomodulatory, anti-inflammatory, and tolerogenic functions. HLA-G antigens play a crucial role in immune suppression at the maternal-fetal interface; they are expressed mainly in first-trimester villous cytotrophoblasts and protect the fetal semiallograft against lysis by maternal natural killer cells. It was reported that HLA expression in the endometrium may play an important role in fertility and embryo implantation, extending their relevance in reproduction beyond the well described expression in extravillous trophoblast at the fetomaternal contact zone during pregnancy (Mika et al., 2018). Results obtained in the primary analysis were refined by subgroup analyses. Specifically, when we stratified patients for number of pregnancy losses, a higher frequency of the HLA-G ins/ins genotype was detected both in women with three or more episodes of pregnancy loss compared with healthy controls with successful pregnancy and in those with two or more episodes. There is evidence that pregnancy complications could be associated with abnormal immunologic interactions at the fetal-maternal interface, since the mother's immune system could consider the fetus as nonself and develop antibodies and cytotoxic T lymphocytes against it (Mosaferi et al., 2013) **.** This immunosuppressive role is believed to be mediated, at least partly, by the HLA-G molecule and polymorphisms in the 3′UTR region of HLA-G, and low or undetectable expression of sHLA-G plays a crucial role in the etiology of spontaneous RPL(Heber et al., 2022). and failure of IVF (Kotze et al., 2013)**.**

5. Conclusions

In in vitro fertilization (IVF), the evaluation of mucin (MUC1), Growth Differentiation Factor-9 (GDF9), and Human Leucocyte Antigen (HLA) may offer important insights into endometrial receptivity. Using these indicators, individualized treatment plans can be created to increase the likelihood of a successful pregnancy by identifying possible fertilization issues.

6. Declarations

Authors' contributions: ZA and RMH designed the experiments, and wrote the manuscript; ZA, RMH , WG performed experiments and collected data, editing and preparing the manuscript for journal submission. ZA, RMH, and WG were checking the final approval of the version to be published. All authors read and approved the final manuscript.

7. Ethical approval

This study was conducted under the ethical principles of the Declaration of Helsinki. The research protocol was reviewed and approved by the Institutional Review Board of the College of Medicine, University of Kerbala D 6/259

in 15/1/2024. In addition, all participants were well- informed about the study's purpose and procedures. Informed consent was obtained from all participants prior to their enrollment in the study.

8. Conflict of interest

The authors declare no conflict of interest.

9. Data availability

Data associated with the current manuscript can be requested from the corresponding author upon reasonable request.

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