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Study the Level of the Pregnancy Associated Plasma Protein-A (PAPP-A) Isolated from Mature Retrieved Oocyte during ICSI Protocol in Iraqi Infertile women

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ABSTRACT

Background: The total number of oocytes, oocyte maturation, and oocyte fertilization all significantly affect how successful in vitro fertilization (IVF) is. Additionally, it has been demonstrated that several chemicals in follicular fluid, including cytokines and growth factors, have a major impact on follicular maturation, ovulation, and follicular atresia. One of these molecules, Pregnancy Associated Plasma Protein-A (PAPP-A), a part of the Insulin like Growth Factor (IGF) pathway, has been demonstrated to be generated by granulosa cells and present in follicular fluid.
Objective: The aim of this study is to measure the level of Pregnancy Associated Plasma Protein-A (PAPP-A) in Follicular Fluid (FF) of infertile women undergoing ICSI, and to looking into the connections of PAPP-A with some reproductive hormones level.
Patients and methods: The study included infertile women from Al-Wazyria Hospital, Infertility and IVF Center, Baghdad (Iraq), from December 2021 to April 2022. This study was conducted on 45 infertile women who are set to start their ICSI cycle.
Results: FF PAPP-A was not significant with hormones level (FSH, LH, E2 and AMH) except PRO hormone was significant.
Conclusion: Outcomes show that no relation between studied reproductive hormones and FF PAPP-A level except PRO levels; it was the main cause from the egg to mature and release from the ovary.
Keywords: Associated Plasma Protein-A, Follicular Fluid, Insulin like Growth Factor, In-vitro fertilization, Case series, University of Baghdad.

INTRODUCTION

A woman's fertility is her biological potential to reproduce based on the monthly likelihood of conception, whereas fertility refers to an individual's ability to replicate itself [(1)]. After 12 months of unprotected sexual activity, a couple is said to be infertile if they are unable to start a pregnancy; about 15% of people worldwide struggle with infertility [(2)].

Female infertility has a wide range of etiologies, including endometriosis, chromosomal abnormalities, lifestyle choices, ovulatory problems, tubal factors, and unexplained infertility. Lifestyle factors have received a lot of attention recently due to the possibility that unhealthy lifestyle choices, such as poor eating habits, stress, drinking, smoking, and obesity, might have a long-term influence on female physiology [(1)].

There is much infertility management that helps to solve the problem by either hormonal treatment or Assisted Reproductive Techniques (ART) such as Intra-Uterine Insemination (IUI), In Vitro Fertilization (IVF) and Intra-Cytoplasmic Sperm Injection (ICSI) [(3)].

Intracytoplasmic sperm injection (ICSI) is a type of assisted reproductive technology used to treat infertility; a single sperm cell is directly injected into the ooplasm during ICSI. ICSI gives a variety of therapy choices, including the use of spermatozoa with weak progressive motility and gametes that were surgically removed from individuals with azoospermia's epididymis and testicles [(4)]. Theca and granulosa cells in the secondary follicle release a fluid that collects in the antral cavity or "antrum," ultimately enveloping the oocyte. This fluid, known as Follicular Fluid (FF), is produced by blood filtration via the theca as well as extra secretions from the theca and granulosa layers. [(5)].

Pregnancy Associated Plasma Protein-A (PAPP-A), a part of the Insulin Like Growth Factor (IGF) pathway, is one of these molecules that has been demonstrated to be generated by granulosa cells and present in FF. IGF-I and IGF-II peptides, their binding proteins IGFBP-1 through IGFBP-6, and Type I and Type II IGF receptors on target cells make up the majority of the IGF system. Through autocrine and paracrine actions, IGFs and IGF receptors are hypothesized to contribute to follicular recruitment, oocyte maturation, and maybe embryo development [(6)].

Pregnancy Associated Plasma Protein-A (PAPP-A) is a zinc metalloproteinase found in the region of the placental syncytiotrophoplast responsible for producing (IGFs). Cleavage of Insulin-like Growth Factor Binding Protein 4 depends on it (IGFBP4). IGFs regulate steroidogenesis, follicular and oocyte development, and other ovarian functions [(7)].

The aim of this study is to measure the level of PAPP-A in FF of infertile women undergoing ICSI, and to looking into the connections of PAPP-A with some reproductive hormones level.

PATIENTS AND METHODS

The study included infertile women from Al-Wazyria Hospital, Infertility and IVF Center, Baghdad (Iraq), from December 2021 to April 2022. This study was conducted on 45 infertile women who are set to start their ICSI cycle. The respondents' ages range from 19 to 45 years.

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Blood samples (5ml) were drawn from each women from vein by disposable syringe on day 2 of the menstrual cycle to measure hormones (FSH, LH, PRO, E2 and AMH) before starting control ovarian stimulation determined by using mini VIDAS system, were put into a plain or gel tube allowed to clot for 30 minutes and then centrifuged at 3000 rounds per minute rpm within 10 minutes to separate the serum, by ELISA. A sterile micropipette was used to transfer (1.5ml) of serum sample into sterile Eppendorf tubes for the next test, which were then frozen at -20 °C until the time of analysis.

FF was obtained from the first retrieved follicle to avoid contamination of blood and flushing medium, and collected in a plane tube. Follicular fluid samples were frozen at -196°C at liquid Nitrogen. Centrifuge at 2500-4000 RPM for approximately 20 minutes. Until the time of the analysis, they were used to evaluate the Follicular fluid PAPP-A level.

A full history has been obtained from each women including: personal history, menstruation history and infertility status. General examination and full infertility investigations were performed on all infertile women. These investigations included husband's seminal fluid analysis, hormonal testing, trans-vaginal ultrasounds, and hystrosalpingography for uterine cavity and tubal patency, and/or laparoscopy for tubal patency and the isolation of Polycystic Ovaries Syndrome (PCO).

Ethical Consent:

This study was ethically approved by the ethics committees of the Biology Department, College of Science, University of Baghdad. (Reference No. CSEC/0122/0052). Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Analysis System- SAS (2018) program. Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and standard deviation (SD), and independent sample t-test was used for comparison between groups. Correlation coefficient was used to measure the correlation between variables in this study [8]. P value ≤0.05 was considered to be statistically significant.

RESULTS

Table 1 summarizes the sociodemographic of the studied women.

Table 1: Results of sociodemographic parameters.

Parameter	Range	Mean ± SD
Age (year)	19.00 – 45.00	33.02 ± 0.85
Infertility period (year)	1.00 – 15.00	5.02 ± 0.52
Weight (kg)	57.00 – 135.00	70.93 ± 1.74
Total no. =45		

Table 2 summarizes the levels of hormones of the studied women.

Table 2: Serum hormonal profile of the infertile women

Hormones	Mean ± SE
FSH (mIU/mL)	7.21 ± 0.82
LH (mIU/mL)	6.85 ± 1.74
Progesterone(ng/mL)	1.37 ± 1.32
E_2 (pg/mL)	1260 ± 48.7
AMH (ng/ml)	2.38 ± 0.72

In the present study, the mean of FF PAPP-A was 2.17 (**Table 3**).

Table 3: Serum and the follicular fluid level of PAPP-A of the studied women.

Marker	Mean ± SE
FF. PAPP-A (IU/L)	2.17 ±0.23

Levels of hormones (FSH, LH, PRO, E2 and AMH) in the serum in day 2 of menstrual cycle and its correlation coefficients with FF PAPP-A value are shown in **Table 4**. The results revealed non-significant correlations between **PAPP-A and** levels of hormones (uterine factor, male factor, ovarian factor, unexplained factor and mixed factor) in the studied women, and a significant correlation with PRO.

Table 4: Correlation coefficients between PAPP-A value and Hormones level

Hormones	PAPP-A FF (IU/L)
FSH (mIU/mL)	-0.14 NS
LH (mIU/mL)	-0.08 NS
PRO (ng/mL)	-0.26 *
E2 (pg/mL)	-0.10 NS
AMH (ng/mL)	0.11 NS

* (P ≤0.05), NS: Non-Significant.

Figures 1, 2, 3, 4 and 5 show the relationship between FF PAPP-A levels and hormones.

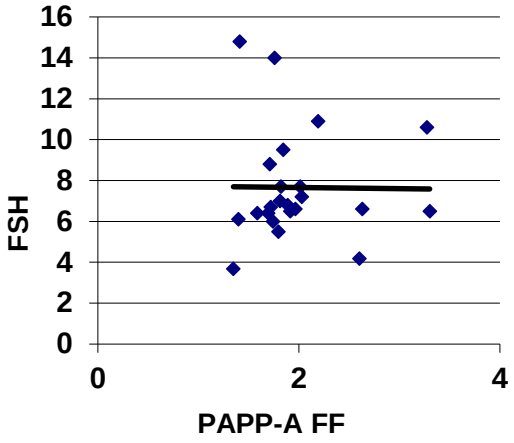

Figure 1: Relationship between PAPP-A FF & FSH

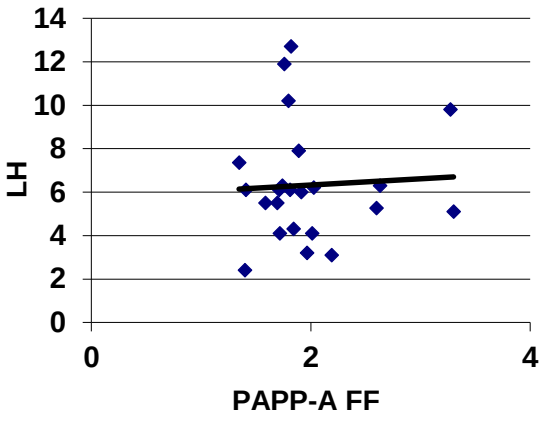

Figure 2: Relationship between PAPP-A FF & LH

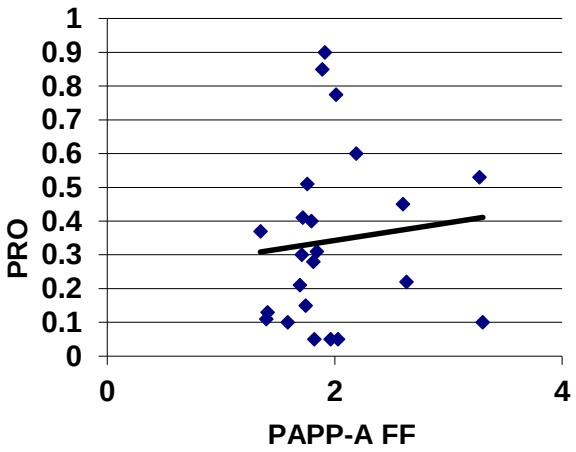

Figure 3: Relationship between PAPP-A FF & PRO

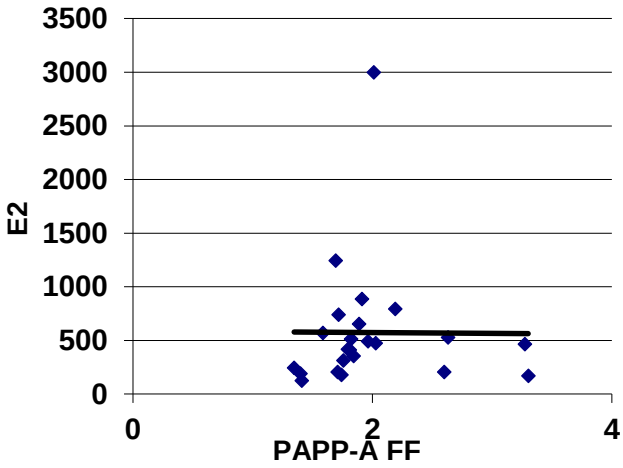

Figure 4: Relationship between PAPP-A FF & E2

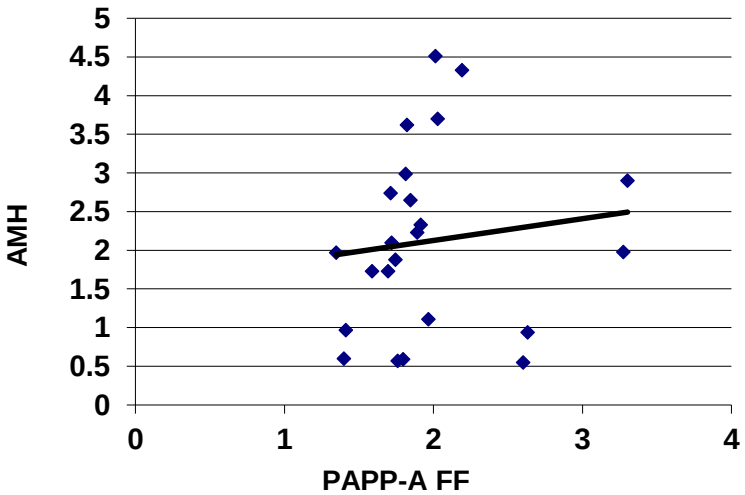

Figure 5: Relationship between PAPP-A FF & AMH

DISCUSSION

Age is considered as one of the most important fertility indicators that has already been identified [9]. As women age, their ovarian egg production gradually declines and the quality of their oocytes declines, which are the two main causes of the loss in female reproductive potential [10]. According to **Dong *et al.*** (2021) theory, when infertility duration lengthens, stress is likely to worsen, perhaps leading to psychological discomfort and sexual dysfunction [11].

Obesity has been associated with a higher chance of developing several illnesses. The most frequent issues include irregular menstrual cycles, an ovulatory infertility, and a pathological pregnancy [12].

According to **Shenta A *et al.* (2020)** [21] mean FSH, LH, PRO and E2 hormones of Iraqi women hormones, aged between 15-29 years were 5.93 (SD 0.42), 0.81 (SD 0.063), 1.25 (SD 0.24), and 128.22 (SD 6.80), respectively; and in age 30-45 years was 7.66 (SD 0.86), 4.37 (SD 1.49), 1.104 (SD 0.18), and 114.81 (SD 4.37), respectively; when we compere this results with our results we found that FSH in 15-29 year was less than our results but it was vary related in age 30-45. LH was reverse, it was less in normal women age 15-29 but related in age 30-45 with our results. PRO have little differences in both ages with our results. E2 have high differences in both ages with our results

Also AMH was 6.494 (SD 11.486) according to **Amer Abed F *et al.* (2019)** this results show high differences than our results of Iraqi infertile women [22].

All this differences between hormones level of healthy and infertile Iraqi women show the great effect of hormones on female fertility.

The mean and range of reproductive hormones vary among infertile women groups, women age, weight and others. Female fertility normally reduces as women age. The ovarian response to stimulation reduces during IVF/ICSI. Some women experience this decline sooner than others. As a result, clinical analysis has been proposed as an early detection method for women with low oocyte quantity and quality. The serum FSH and LH concentrations on day 2 of the menstrual cycle are extensively used as a marker of ovarian reserve in women undergoing fertility assessment [13].

Women's high FSH levels may protect the following cohort's 2-5-mm follicles from atresia, leading to the follicles to become bigger throughout the interovulatory period. Despite the possibility of modest ovarian insufficiency suggested by high estradiol levels in the early follicular phase, functional cysts found at this time may contain competent oocytes [18].

In accordance with the fact that hormone levels vary depending on the menstrual phase, the majority of variables were notably rhythmic during the follicular phase but not the luteal phase of the menstrual cycle. These findings have important implication for our knowledge of how reproductive function is regulated. Our findings demonstrate that depending on the menstrual period, the circadian clock controls female reproductive hormones differently in humans [16].

Hormone changes often endure for several years and are mild, overlaid upon progressively increasing cycle irregularity, and for this reasons hormones effect directly on female fertility [17].

The drop in PAPP-A levels in ART pregnancies adds to the scientific proof that ART and non-ART pregnancies differ; it might have effects that go beyond the combo screen. PAPP-A is a growth-stimulating IGF binding protein cleaver. PAPP-A levels in ART pregnancies might simply be a predictor of these issues, which are more prevalent in the ART numbers. PAPP-A levels have also been connected to negative pregnancy outcomes (hypertension, pre-eclampsia, and gestational diabetes) and perinatal outcomes (prematurity, low birthweight, and neonatal death) [10]. Although PAPP-A levels were high in the ovarian follicles of women undergoing IVF, previous study found that intrafollicular PAPP-A did not significantly increase blood PAPP-A concentrations.

Jepsen *et al.* (2016) have demonstrated that intrafollicular concentration of PAPP-A rises in human antral follicles following treatment of hCG, which supports these expression results [24].

There were non-significant results between PAPP-A with FSH, LH, E2 and AMH, while PRO was significant.

This agrees with **Durdağ *et al*. (2019)** and **Bøtkjær *et al*. (2015)**, but disagrees with **Mazerbourg and Monget (2018)**, Ovarian PRO secretion is regulated by both FSH and LH in women undergoing controlled ovarian stimulation COS with exogenous gonadotropins, with LH activity becoming more essential as the follicle grows into the preovulatory stage [15,19,20].

Amor *et al.* (2009) [10] suggest a hypothesis in which hormone therapy during embryo transfer causes aberrant levels of ovarian steroid hormones and other components yet to be discovered, resulting in a decrease in PAPP-A synthesis. Although PAPP-A is produced by the placenta, Because the impact is observed for hormone treatment delivered before to implantation and development of a placenta, presumably indicating decreased early implantation with some kinds of ART, the drop in PAPP-A is most likely mediated by a hormonal action on the endometrium, Lower secretion of PAPP-A.

Ovarian PRO secretion is regulated by both FSH and LH in women undergoing controlled ovarian stimulation COS with exogenous gonadotropins, with LH activity becoming more essential as the follicle grows into the preovulatory stage [19].

Circulating PRO's commonly recognized effect on circulating LH concentration may explain the effect of circulating PRO on the kinetics of the follicular process of divergence. Increased PRO, for example, lowered the frequency of (GnRH) pulses, leading in a reduction in the frequency of LH pulses. Furthermore, a raise in circulating PRO can operate of LH release in response to GnRH administration. In their earlier research, they were unable to distinguish between the effect of raised PRO and the potential contribution of circulating LH to

the follicular variation process since, during the second follicular wave, elevated circulating PRO was accompanied by reduced LH [23].

In our expect hormones control the metabolism and growth of ovarian, uterine, and placental tissues, they are essential for the healthy operation of the female reproductive system. Therefore, any flaw might make a woman infertile or subfertile. Menstrual/estral irregularity, anovulation, abortion, premature birth, preeclampsia, intrauterine growth restriction, postpartum thyroiditis, and child mental impairment are further well-documented effects of maternal thyroid dysfunction.

CONCLUSION

There were non-significant correlations between all studied fertility hormones (FSH, LH, E2 and AMH) except PRO was significantly increase with FF PAPP-A.

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