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The Relationship between the Level of the Pregnancy Associated Plasma Protein-A (PAPP-A) and Some Reproductive Hormones in Serum of Iraqi Women Undergoing ICSI Protocol

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ABSTRACT

Background: Pregnancy Associated Placental Protein-A (PAPP-A) is a zinc-binding metalloproteinase with a key role in the insulin-like growth factor (IGF) pathway. The IGF system plays an essential role in follicular development, including steroid hormone synthesis, growth, recruitment, and apoptosis of follicles of women undergoing intracytoplasmic sperm injection (ICSI).

Objective: The objective of this study is to measure Pregnancy Associated Plasma Protein-A (PAPP-A) in serum of infertile women undergoing ICSI, and to investigate the association of PAPP-A with some reproductive hormones level. A total of 45 infertile women.

Results: The results of this study was Serum PAPP-A level was not significant with hormones level (FSH, LH PRO and AMH) except E2 hormone was significant.

Conclusion: Outcome show that no relation between studied reproductive hormones and serum PAPP-A except E2 levels that are high cause the egg to mature and release from the ovary.

Keywords: PAPP-A, E2, Infertility and hormones.

INTRODUCTION

Infertility is described as the inability to get pregnant after 12 months of sexual activity, and it affects 15%-17% of couples globally, with around 50% of them being connected to female infertility problems like (risk factor and known causes, environmental factors, weight changes, age and life style and ovarian and hormonal function problems) [(1)].

There is many infertility management that help 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) [(2)].

Intracytoplasmic sperm injection (ICSI) is a technique of assisted reproductive technology used to treat infertility, a single sperm cell is directly injected into the ooplasm during ICSI. ICSI provides a variety of therapy options, involving the use of spermatozoa with slow progressive motility and gametes surgically extracted from the epididymis and testicles of those with azoospermia [(3)].

Pregnancy-Associated Plasma Protein A (PAPP-A) is a glycoprotein and a highly selective metalloproteinase that attaches to glycosaminoglycan's on the cell surface and associated in the cleavage of IGFBP, specifically IGFBP4,5, resulting in an increase in bioactive IGF. PAPP-A also acts as a growth-promoting enzyme within the tissue, producing bioactive IGF when it comes into contact with IGF receptors [(4)].

Pregnancy Associated Plasma Protein-A (PAPP-A) is found in little amounts in the serum of males and non-pregnant females, but at rise concentrations in the serum of pregnant females [(5)].

Last studies expression that low expression of PAPP-A increased the risks, PAPP-A in normal women before pregnant is lower than infertile women, and after get pregnant the PAPP-A level be higher in normal women than in infertile women [(6)].

SUBJECTS AND METHODS

The sample used to design this study was infertile women from the Al-Wazyria Hospital, the Infertility and IVF Center, Baghdad / Iraq, from December 2021 to April 2022. This research has 45 infertile women who are set to start their ICSI cycle. The respondents' ages range from 19 to 45 years.

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 (bio Merieux , France), and in one day before oocyte retrieval to measure PAPP-A in serum, 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. About (1.5ml) of serum sample was transferred by sterile micropipette into sterile Eppendorf tubes for following test and kept frozen at -20 °C until time of analysis.

A thorough medical history, gynecological and 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 laproscopy for tubal patency and the isolation of Polycystic Ovaries Syndrome (PCO).

Ethical consent:

A full history has been obtained from each women including: personal history, menstruation history and infertility status. All of them were made aware of the study's subject and signed a written

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consent form. The patients' assent was obtained, and the study was approved by the ethics committees of the Biology Department, College of Science, University of Baghdad. (Reference No. CSEC/0122/0052).

Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of correlation coefficient between variables in this study [7].

RESULTS

The age, infertility period and weight was expressed in (mean ± standard error). Median infertility time of the patients was 5 (1- 15) years. The mean of Age was (33.02 ±0.85 year) and mean of Weight was (70.93 ±1.74 Kg). As show in **table 1**

Mean ±SE was measured to Levels of hormones (FSH, LH, PRO, E2 and AMH) in the serum in day 2 of menstrual cycle, FSH (7.21 ±0.82), LH (6.85 ±1.74), PRO (1.37 ±1.32), E2 (1260.00 ±438.7) and AMH (2.38 ±0.72) respectively. And range of hormones was FSH (3.67-14.80), LH (1.32-11.90), PRO (0.05-3.00), E2 (51.90-3000.00) and AMH (0.55-4.87) as show in **table (2).**

In the present study, there was descriptive statistics were expressed in mean ± standard error and range. Mean ± standard error of serum PAPP-A was (3.07 ±0.39 IU/L), and range of serum PAPP-A was (1.71-5.25 IU/L), as show in **table (3).**

Levels of hormones (FSH, LH, PRO, E2 and AMH) in the serum in day 2 of menstrual cycle and its correlation coefficients with serum PAPP-A value are shown in **table (4).** The results revealed that a non-significant ($p>0.05$) difference in levels of hormone in all studied groups as fallowing FSH (0.02), LH (0.04), PRO (-0.15) and AMH (0.02) respectively. While the E2 hormone level was significant differ with the level of the serum PAPP-A (0.25).

Table (1): General characteristics of the infertile women

PARAMETER	RANGE	MEAN ±SE
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

Table (2): Serum hormonal profile of the infertile women.

Hormones	Mean ± SE	Range
FSH (mIU/mL)	7.21 ±0.82	3.67-14.80
LH (mIU/mL)	6.85 ±1.74	1.32-11.90
Progesterone(ng/mL)	1.37 ±1.32	0.05-3.00
E_2 (pg/mL)	1260.00 ±438.7	51.90-3000.00
AMH (ng/ml)	2.38 ±0.72	0.55-4.87

Table (3): Serum level of PAPP-A of the studied women

Marker	Mean ± SE	Range
S. PAPP-A (IU/L)	3.07 ±0.39	1.72-5.26

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

Hormones	serum PAPP-A (IU/L)
FSH **(mIU/mL)**	0.02 NS
LH **(mIU/mL)**	0.04 NS
PRO **(ng/mL)**	-0.15 NS
E2 **(pg/mL)**	0.25 *
AMH **(ng/mL)**	0.02 NS

* ($P \leq 0.05$), NS: Non-Significant.

The next figures (1, 2, 3, 4 and 5) show the relationship between serum hormones levels and serum PAPP-A levels.

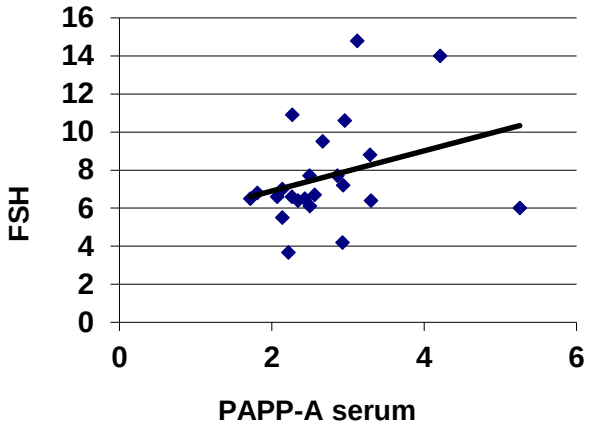

Figure 1: Relationship between PAPP-A serum & FSH

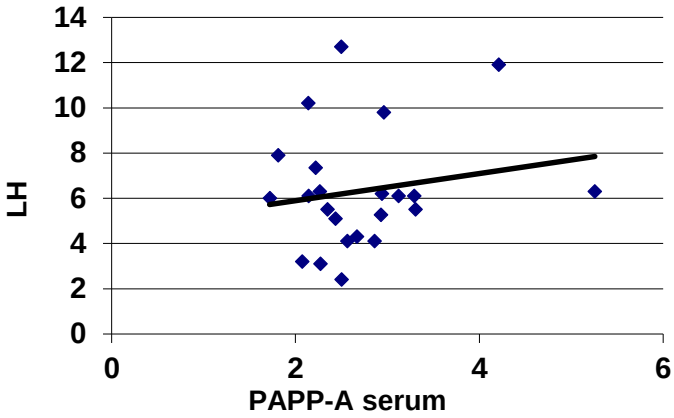

Figure 2: Relationship between PAPP-A serum & LH

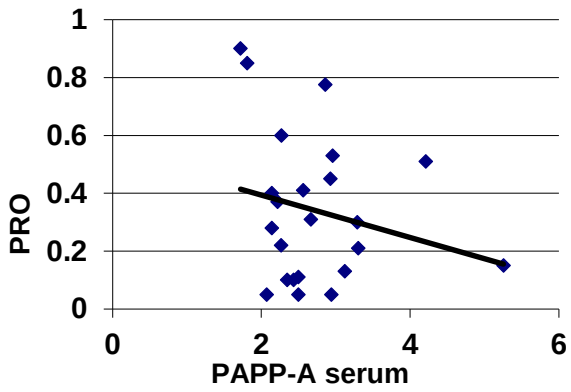

Figure 3: Relationship between PAPP-A serum & PRO

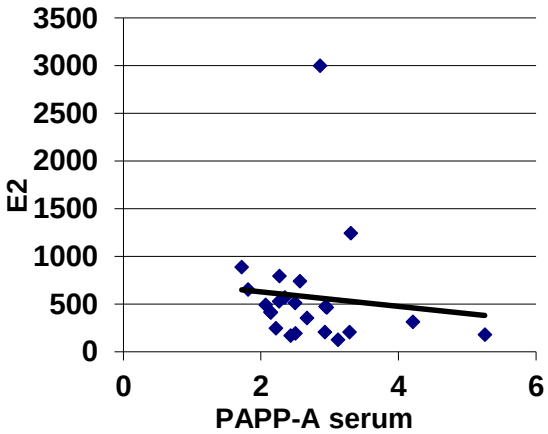

Figure 4: Relationship between PAPP-A serum & E2

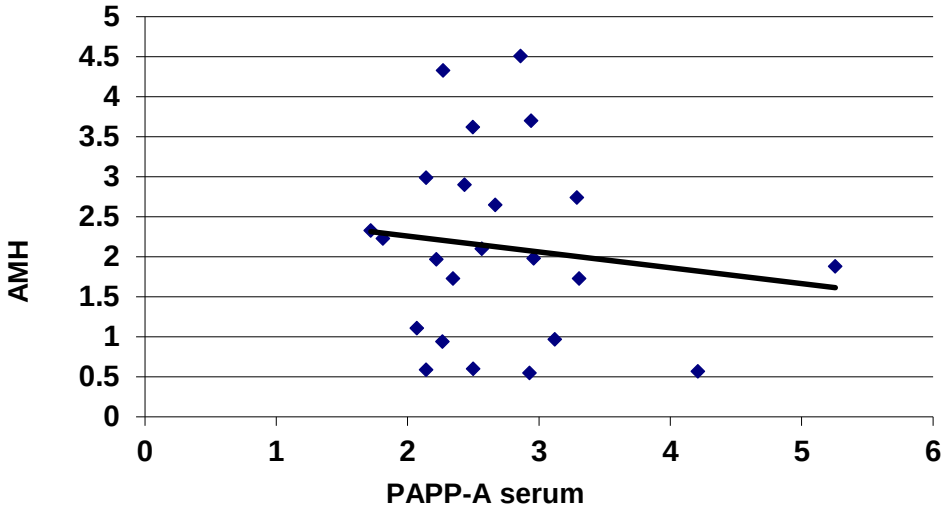

Figure 5: Relationship between PAPP-A serum & AMH

DISCUSSION

Age considered one of the most important fertility indicators that has already been identified [8]. 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 [9].

Dong *et al.* (2021) hypothesis is that when the duration of infertility increases, stress is likely to develop even more, perhaps aggravating the situation to psychological distress and causing sexual dysfunction.

Obesity has been linked to an increased risk of certain disorders. Menstrual cycle problems, an ovulatory infertility, and a pathological state during pregnancy are the most prevalent [11].

According to Shenta A *et al.,* the Mean ± SE of Iraqi women hormones, age between 15-29 years (FSH, LH, PRO and E2) was 5.93±0.42, 0.81±0.063, 1.25±0.24 and 128.22±6.80 respectively; and in age 30-45 years was 7.66±0.86, 4.37±1.49, 1.104±0.18 and 114.81±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±11.486 according to Amer Abed F *et al.,* this results show high differences than our results of Iraqi infertile women.

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

The mean ± SE and range of reproductive hormones are variances 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 females 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 [14].

Because hormone levels differ based on the menstrual phase, the majority of variables were significantly rhythmic during the follicular phase but not the luteal phase of the menstrual cycle. These discoveries have significant implications for our understanding of how reproductive function is controlled. Our findings show that the circadian clock governs female reproductive hormones differently in humans depending on the menstrual cycle [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. [5].

PAPP-A primary function is to promote cell development by cleaving inhibitory IGFBPs, particularly IGFBP4, resulting in increased IGF bioavailability. It is the only single proteinase for IGFBP4 and is connected to the cell surface by glycosaminoglycans. According to research, PAPP-A has been found in a variety of cells, including the ovarian granulosa cell, osteoclasts, and fibroblasts surrounding adipose tissue, as well as vascular smooth muscle cells [22]. So PAPP-A before pregnancy is small amount and mainly secrated not from placenta (the mine secretion source of PAPP-A after pregnancy).

Levels of PAPP-A are also linked to undesirable pregnancy outcomes (high blood pressure, pre-eclampsia, and gestational diabetes) and pregnancy consequences (prematurity, low birthweight, and neonatal mortality) [5].

There was non-significant results between FSH, LH, PRO and AMH with serum PAPP-A. While E2 was significant. The significant relationship between E2 and PAPP-A this agree with Bøtkjær *et al.*,. According to research, low maternal serum PAPP-A levels throughout pregnancy are not linked to peak E2 levels on the trigger day. Previous research has revealed that IGF signaling is critical for steroid synthesis in human follicle development, possibly via PAPP-A control [18].

Despite differences in follicle wave dynamics, the relevance of the FSH high point in beginning the follicular wave, the function of the FSH low, and variations in granulosa cell responsiveness to LH during the deviation process remain central to contemporary follicle selection models [23].

Fortune *et al.,* demonstrate that after the dominant follicle is identified as being bigger than the remainder of the cohort, its FF has much greater quantities of estradiol and PAPP-A. And they found that the rise in estradiol might potentially have an effect negative feedback on FSH production to inhibit the acquisition of further follicles in the cohort PAPP-A.

The significant relationship between E2 and PAPP-A it may be because appears to be more essential than previously considered. Estradiol's primary role in a woman's body is to encourage the growth and development of her reproductive system. Estradiol levels

that are high cause the egg to mature and release from the ovary as well as the uterine lining to thicken, which facilitates the implantation of a fertilized egg during pregnancies.

The AMH was non-significant with serum PAPP-A and this results was agree with Bøtkjær *et al*., and disagree with Durdağ *et al*.,. AMH has an important role in regulating ovarian folliculogenesis. Granulosa cells in ovarian follicles manufacture it. Because of its significant relationship with the number of antral follicles, the AMH serum level is now frequently used as a measure for ovarian reserve and as a predictor of reproductive potential (Homburg and Crawford, 2014). For example, as women age, AMH levels fall, indicating a limited ovarian reserve [(25)], which may explain why a high level of AMH is related with a greater pregnancy rate.

Moreover, the lowered PAPP-A is connected to hormone stimulus and the female's reaction to hormone therapy. PAPP-A levels were normal in normal pregnancies when no hormone therapy was taken. Amor *et al.,* suggest a hypothesis in which hormone therapy during embryo transfer causes aberrant levels of ovarian steroid hormones and other undisclosed components, As a result, PAPP-A production is reduced. Although the placenta generates PAPP-A, the impact is most likely mediated by a hormonal action on the endometrium, as the impact is evident for hormone therapy delivered prior to implantation and placenta establishment, presumably indicating decreased early implantation with some kinds of ART. Lower PAPP-A secretion, the decrease in PAPP-A levels in ART pregnancies adds to evidence that ART pregnancies vary from non-ART pregnancies, which might have consequences beyond the combination screen. PAPP-A is an insulin-like growth factor binding protein cleaver that promotes growth [(5)].

For the first time in Btkjaer's knowledge, displays PAPP-A was found in the theca cell layer of microscopic antral follicles 4-6 mm in diameter, together with substantial AMH staining in the GC layer. PAPP-A expression moved to the GC layer, with the highest levels in mature follicle GCs, but AMH expression ceased in follicles larger than 8-9 mm in diameter [(18)].

Amor *et al.,* looked at the effect of hormone and no hormone therapy getting, regardless of whether the embryos were fresh or frozen-thawed, we discovered that transfer cycles with any hormone treatment resulted in lower PAPP-A levels than those without.

Hormones, as we know, are essential for the proper functioning of the female reproductive system because they govern the metabolism and development of ovarian, uterine, and placental tissues. As a result, any anomaly may result in infertility or subfertility in women. Anovulation, abortion, preterm delivery, preeclampsia, intrauterine growth restriction, postpartum thyroiditis, and mental damage in children are all well-documented effects of maternal thyroid dysfunction.

CONCLUSION

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

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