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ORIGINAL ARTICLE

The Impact of Pregnancy Associated Plasma Protein-A (PAPP-A) on Live and Dead Embryos at Various Developmental Stages

Zainab Omar Yousif*
National Diabetes Center, Mustansiriyah University, Baghdad, Iraq

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ABSTRACT

Pregnancy Associated Placental Protein A (PAPP-A) is Zinc-Binding metalloproteinase with a vital role in insulin like growth factor (IGF) pathway. IGF system plays a key role in follicular development, including steroid hormone synthesis, growth, recruitment, and apoptosis of follicles. There is minimal information in the literature regarding PAPP-A levels in Follicular Fluid (FF) of women that undergoing intra-cytoplasmic sperm injection (ICSI). The purpose of study to detect PAPP-A in blood and follicular fluid during oocyte pick up in infertile women undergoing ICSI, and to investigate the role of PAPP-A in embryonic development. The findings showed a nonsignificant relationship between total oocyte number and oocyte maturity and serum and FF PAPP-A, and fertilization rate. There was a significant correlation between live and dead embryos with different embryonic stages. There was a non-significant correlation between serum and FF PAPP-A with total, mature, and fertilized oocytes, and the oocyte quality parameters examined in this study (total oocyte, mature oocyte, and fertilized oocyte) were influenced by numerous factors (age, prior pregnancies, weight, and others). Live and the dead embryo was not-significant in the embryonic stages (two-cell stage, four-cell stage, and eight-cell stage embryos), except two cell stage dead embryo with serum PAPP-A and four-cell stage dead embryo with FF PAPP-A was high significant differences.

Corresponding Author:

* Zainab Omar Yousif
National Diabetes Center, Mustansiriyah University, Baghdad, Iraq
Email: zainabomar@uonialmustansiriyah.com

1- INTRODUCTION

The ovary is an organ that produces mature oocytes and hormones to control reproductive activities. The ovary houses the oocyte reserve, which produces mature eggs by processes called oogenesis for the rest of the reproductive lifetime, which ends with menopause and the termination of ovulation. The loss of ovarian function is followed by a reduction in gonadal hormones [1, 2]. The process by which Primary Sex Cells in Females mature into fully developed gametes in female ovaries is known as oogenesis. The embryo's yolk sac wall and the allantois contain primary germ cells, or "gonocytes," which undergo a two-step process, maturation of the nucleus and the cytoplasm [3, 4].

Infertility is the fail to have a pregnancy after 12 months (one year) of sexual activity, which affects 15% to 17% of couples global. 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) and this problem can treating by many techniques [5]. There is several infertility management that helps to fix the problem by either hormone medication or assisted reproductive procedures (ART), such as In Vitro Fertilization (IVF), Intra Uterine Insemination (IUI) and Intra Cytoplasmic Sperm Injection (ICSI) [6, 7].

Assisted reproductive technology ART is define as "any procedure that involves handling sperm and oocyte outside of the body to trigger pregnancy"." In general, this procedure includes Controlled Ovarian Stimulation (COS), retrieval of oocytes, IVF, and embryo or embryo transfer into the uterus. Emergency physicians should treat these patients equally, even though many women conceive with just ovarian stimulation, which does not quite fall within the CDC's definition of ART [8, 9]. Pregnancy Associated Plasma Protein-A PAPP-A is a zinc-binding metalloproteinase that plays a key part in the (IGF) pathway. The IGF structure is critical for follicular development, including steroid hormone production, follicle growth, recruitment, and apoptosis. A frequent endocrine condition typically associated with insulin resistance and increased ovarian hyperandrogenism and hyperinsulinemia, resulting in poor follicular growth and oocyte maturation [10]. However, it was observed that PAPP A in the Follicular Fluid not used to estimate the quality of the embryo and fertilization. It also suggested that PAPP A level might indicate follicular maturity but not predict ovum reproductive Possibility. PAPP-A levels might reveal follicular maturity but did not predict ovum fertility potential [11].

The study aims to detect PAPP-A in serum and follicular fluid of woman during oocyte pick up in infertile women undergoing ICSI, and to investigate the role of PAPP-A in different embryonic development.

2- MATERIALS AND METHODS

The sample utilized to create this study was Female infertility from the Al-Wazyriah Hospital. Infertility and IVF Center, Baghdad / Iraq, from December 2021 - April 2022. This research has 45 Female infertile who are set to start their ICSI cycle. The respondents' ages range from 19 - 45 Years. Blood samples (5ml) were extracted from each women from vein by disposable syringe in one day before oocyte extraction to test PAPP-A in serum, were put into a plain or gel tube allowed to clot for 30 minutes and then centrifuged at 3000 rpm within 10 minutes separate the serum, by ELISA. For the subsequent test, a sterile micropipette was used to transfer about 1.5 ml of serum sample to sterile Eppendorf tubes, where it was frozen at -20 °C until analysis. The FF was obtained from 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 in 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 [1].

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).

2.1 Statistical analysis

The Chi square test was utilized to significantly compare percentages (0.05 – 0.01 probability), and the statistical analysis system SAS (2018) program was utilized to identify the impact of different components in the study parameters. The study's estimate of the correlation coefficient was between the variables [12].

3- RESULTS AND DISCUSSION

The age, infertile period and weight was represented in (mean ± standard error). Median of infertile time was (5) (1-15) Years. The average weight was 70.93 ±1.74 kg, and the average age was 33.02 ±0.85 years. As show in table 1.

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

A deterioration in the quality of produced embryos caused by aged oocytes, as well as an older uterus and hormonal disruption caused by increased maternal age, resulting in lower ICSI/IVF pregnancy success rates [13].

Dong *et al.* (2021) [14], Hypothesis that stress is likely to escalate as the length of infertility grows, perhaps exacerbating the situation to psychological discomfort and producing sexual dysfunction. The endocrine system, which is in charge of most essential bodily functions, particularly the female reproductive system, is impacted by weight and an unhealthy lifestyle. Hormonal secretion from the endocrine system causes many defects in the female reproductive system, including oocyte production, maturation, fertilization, implantation, and a host of disorders.

The total quantity of oocytes taken from the women ranged between 1 – 27 (median 8) the mature oocytes range was 0 – 20, the median was 6, and fertilized oocytes range was 0 – 15 Median 5, as shown in table 2.

Table (2): Numbers of retrieved oocytes, their maturation, and fertilization of the infertile women

Oocytes	Median	Range
Total number of oocyte	8.00	1.00-27.00
Mature oocytes	6.00	0.00-20.00
Fertilized oocytes	5.00	0.00-15.00

Ovarian stimulation, follicular development monitoring, oocyte harvesting, sperm preparation and insemination, embryo culture, embryo transfer, and luteal support are all part of the process. The number of recovered oocytes and the success rate of ART can be significantly impacted by other parameters, particularly feminine age. The number of embryos obtained is related to the number of oocyte obtained. To boost the quantity of retrieved oocytes, researchers recommend follicular ambition followed by a single 2 mL flush. Double lumen needles and flushing produced 20% more oocytes. Some investigations indicated no change in the number of oocyte harvested, fertilization %, the quality of the embryo, or the pregnancy rate. Others claim that aspiration without flushing reduces the amount of anesthesia required and saves surgical time [15]. Furthermore, previous research found a significant immune-expression of PAPP-A in GCs from preovulatory follicles, as well as a substantial positive connection relationship FF PAPP-A levels and follicular size. Therefore, the existence of enhanced PAPP-A appearance in larger pre-ovulatory follicles validates and extends prior findings [16].

In this results that PAPP-A level did not play an important effect on the total number of maturing oocyte and fertilized oocyte, In contrast to its function in early follicular development. This agreement with Durdağ *et al*., 2019 [6] found that PAPP-A values showed no discernible differences in the follicular fluid of high-quality embryos. This demonstrated that the PAPP-A relationship refused to play an essential role in maturing oocyte and embryo expansion as it did in early follicular growth. This result confirmed the results obtained by (Pongsuthirak *et al.*, 2018) [17], They stated there was no significant difference in maturity rate, fertilization rate, or the rate of formation of good-quality blastocysts in oocytes collected from pregnant and non-pregnant women. A non-significant ($p>0.05$) difference was found in the results. Between PAPP-A in serum and FF and a total number of oocytes, mature oocyte and fertilized oocyte, as shown in table (3).

Table (3): The correlation analysis between serum and FF PAPP-A levels with numbers of retrieved oocytes, maturation, and fertilization of the infertile women

Oocyte	S. PAPP-A (IU/L)		FF. PAPP-A (IU/L)	
	R	**P**	**R**	**P**
Total number of oocyte	-0.07	0.62 NS	0.19	0.20 NS
Mature oocytes	-0.03	0.84 NS	0.23	0.12 NS
Fertilized oocytes	-0.09	0.52 NS	0.24	0.10 NS
Correlation (P), correlation coefficient (R).				

Firouzabadi *et al*., (2012) [18], demonstrated that PAPP A in the FF could not be used to forecast fertilization and embryo quality.

Our results suggest that it is not possible to predict which oocytes will be fertilized based on low FF concentrations in PAPP A ovarian follicles. The impact of FF levels PAPP A on the results of oocyte fertilization and the

subsequent development of embryos, and this disagrees with Wang *et al.* (2006) [19]. In contrast, Stanger *et al*. (1985) [20] stated that PAPP A levels might indicate follicular maturation but did not expect ovum fertility possibility. However, it appears to support Firouzabadi *et al*., (2012) [18] and Stanger *et al*. (1985) [20] findings the variation might be explained by differences in sample sizes and embryo numbers used in the research. Therefore according to the size samples of our study, we could find that the effect of the PAPP-A level in both serum and FF may not be a direct factor in assessing the quality of the oocytes and successful of infertilization.

As shown in table (4) demonstrated median and range of living and dead in different embryonic stages. There was a significant correlation between all embryonic developmental stages (Two-cell stage, Four-cell stage and Eight-cell stage) with live and dead embryos.

Table (4): compares dead and live embryos in different embryonic developmental stages

	Dead embryo Median (Range)	**Live embryo Median (Range)**	***P* value**
Embryo stage			
Two-cell stage	0.00 (0.00-2.00)	5.00 (0.00-15.00)	0.00 *
Four-cell stage	0.00 (0.00-4.00)	5.00 (0.00-15.00)	0.00 *
Eight-cell stage	0.00 (0.00-4.00)	5.00 (0.00-13.00)	0.00 *

When differentiated sperm and oocytes fertilize, a totipotent zygote is created. This zygote undergoes a number of reprogramming and alterations to become pluripotent, marking the beginning of the early phases of embryonic growth. The zygote, following the fertilization of two separate highly differentiated gametes, initiates its early embryonic development, which is essentially a cell reprogramming process [21].

Embryos live or dead, have a significant correlation with embryo stages. This may-be because embryo cleavage is fast, and all this cleavage is in vitro and controlled by the technical embryologist, so it's little abnormal conditions may kill or enhance embryo development.

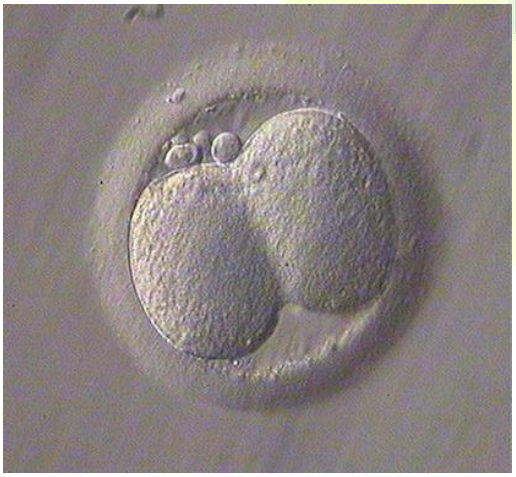

Figure (1): Two-cell stage embryo

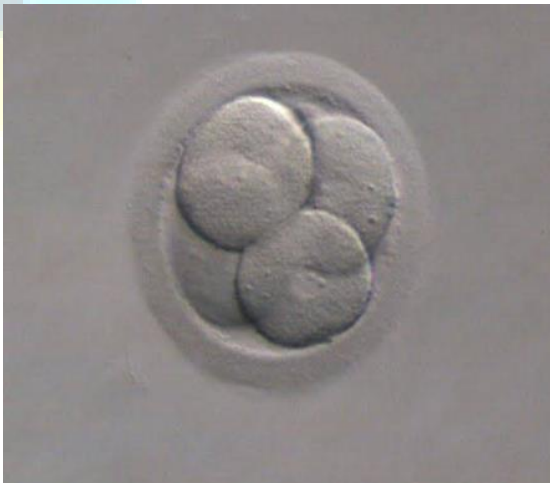

Figure (2): Four-cell stage embryo

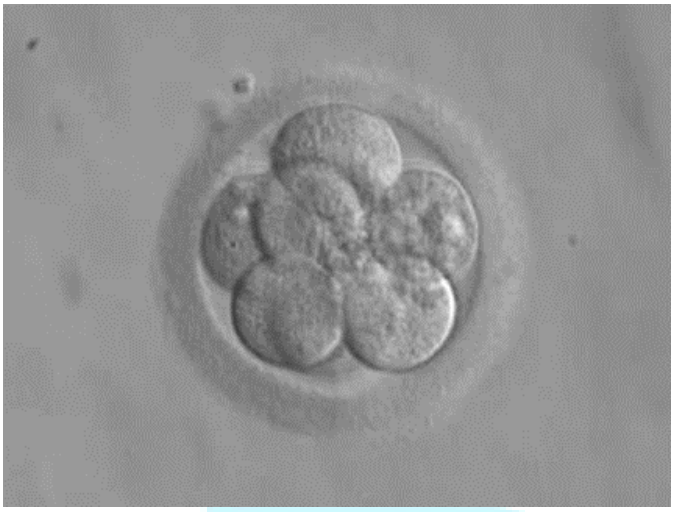

Figure (3): Eight-cell stage embryo

Though there was no significant association between the FF levels of PAPP-A and embryos development, this agree with Wang *et al.* (2006) [19], discovered that FF from follicles, which contains fertilized eggs, has PAPP A levels. Showed the developmental stoppage of embryos on the second day was much lower compared to those from follicles in which oocytes could not be fertilized. However, our findings showed a highly significant correlation between serum PAPP-A and two-cell stage dead cells, which is consistent with earlier findings in this study. Increased serum PAPP-A in pregnant women compared to non-pregnant women may be due to the interaction between PAPP-A and hormones that regulate cell division. While we found a delay effect of FF PAPP-A on the developmental stage, when the dead embryos were at four-stage of development.

The correlation coefficients in PAPP-A in serum and FF and compared with live and dead oocyte in three stages (Two cell-stage, four cell-stage and eight cell-stage) are show in the table 5. The outcomes show that highly significant (P≤0.01) in dead Two cell-stage with serum PAPP-A, significant (P≤0.05) in dead four cell-stage with FF PAPP-A, and non-significant for the rest of results as shown respectively, (-0.12, -0.13, 0.02, -0.05, 0.11, -0.02, 0.07, 0.06 and 0.07).

Table (5): The relationship analysis between serum and FF PAPP-A levels with dead and live embryos in different embryonic developmental stages

PARAMETER	EMBRYO STAGES					
	Two cell-stage		Four cell-stage		Eight cell-stage	
	Live	Dead	Live	Dead	Live	Dead
SERUM PAPP-A	-0.12 NS	0.36 **	-0.13 NS	0.02 NS	-0.12 NS	-0.05 NS
FF PAPP-A	0.11 NS	-0.02 NS	0.07 NS	0.25 *	0.06 \NS	0.07 NS
* (P≤0.05), ** (P≤0.01), NS: NON-SIGNIFICANT.						

Improved Maturation of oocytes and early development of embryos are connected to reduced FF levels of PAPP A and increased levels of IGF-II, IGFBP-3, and IGFBP-4 in ovarian follicles, according to Firouzabadi et al., (2012) [18], and these factors may be exploited in predicting which oocytes would be successfully fertilized and grown into early embryos. According to our findings, superior embryo quality and oocyte fertilization cannot be predicted by PAPP-A FF levels.

It is evident that the PAPP-A levels of the patient's two follicles did not differ statistically significantly, and that the PAPP A value of one follicle possibly considered an aggregate of the patient's follicular fluid. Additionally, follicular PAPP-A values were associated with beginning pregnancy rather than embryo development, according to comparable PAPP-A of FF from which various quality embryos are produced. [6].

Proteo-lysis of IGFBP-4 by PAPP A would only, have a modest if any, impact on oocyte maturity and succeeding embryonic development, as opposed to the stimulating effect in early follicular expansion previously described [22, 23].

4- CONCLUSION

PAPP-A levels in blood and follicular fluid had no significant correlation with oocyte quantity, maturity, or fertilization, demonstrating that PAPP-A is not a reliable indicator of oocyte quality in ICSI cycles. However, several connections with early embryonic demise point to a restricted involvement in embryo development. Further research with bigger samples is required to establish its potential therapeutic value.

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