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**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/5794
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/5794>



RESEARCH ARTICLE

A STUDY OF SERUM AND FOLLICULAR FLUID C. REACTIVE PROTEIN AND ANTISPERM ANTIBODY AS PREDICTIVE MARKERS FOR HUMAN OOCYTE AND EMBRYO QUALITY AND ICSI OUTCOME.

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Manuscript Info

Manuscript History

Received: 07 September 2017
 Final Accepted: 09 October 2017
 Published: November 2017

Key words:-

C. Reactive Protein, Anti-sperm antibodies, Follicular Fluid, Oocyte quality, Embryo Quality, ICSI.

Abstract

Background: C. reactive protein (CRP) is an acute phase reactant. Its level increases in infection, inflammation and following hormonal stimulation. While antisperm antibodies (ASA) cause immunological infertility through their affect on motility and capability of fertilization of spermatozoa which may negatively affect embryo development & implantation

Objectives: To determine the predictive value of C. reactive protein (CRP) & Antisperm antibodies (ASA) detection in serum and follicular fluid for assessment of oocyte quality, embryo quality and ICSI outcome in patients using different stimulation protocols.

Materials and Methods: Fifty eight (58) infertile women undergoing intra-cytoplasmic sperm injection cycles with an age of (21-41) years old were included in this study. C. reactive protein was measured in serum and follicular fluid by using agglutination and serial dilution method. Also an estimation of antisperm antibodies (ASA) levels in serum and follicular fluid was done by using ELISA.

Results: The results of this study showed a significant negative correlation between the C. reactive protein & Anti-sperm antibodies levels in serum and follicular fluid with oocyte and embryo quality in all patients. Eighteen women (31%) achieved pregnancy (pregnant group), while forty women (69%) failed to achieve pregnancy (non-pregnant group). A significant difference was found in the concentration of C. reactive protein and antisperm antibodies in both serum and follicular fluid ($P < 0.05$) between pregnant and non-pregnant groups.

Conclusion: Serum and Follicular Fluid C. reactive protein and Anti-sperm antibodies are good predictors for oocyte quality, embryo quality and pregnancy rate in women undergoing IVF/ICSI cycles.

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Introduction:-

Infertility is a complex condition with important psychological, monetary, demographic and medical implications¹. It is defined as failure to achieve pregnancy within 1 year of unprotected intercourse. This condition may be classified as primary infertility, in which no previous pregnancies have occurred, and secondary infertility, in which a prior pregnancy, although not necessarily a live birth, has occurred². The causes of infertility may be classified into female factor, male factor, and unexplained infertility⁵.

Assisted reproductive technologies (ARTs) with various treatment protocols for ovarian stimulation developed to be used in infertility treatment. These protocols are aimed at the development of multiple instead of one dominant follicle and there by result in a non-physiological condition³. A woman ovarian response to stimulation drug is mainly determined by her ovarian reserve, which can be defined as the number and quality of the remaining follicles and oocytes in both ovaries at a given age⁴.

CRP is a pentameric protein synthesized by liver hepatocytes in response to acute stimuli. Its half-life in the circulation is ~19 hr, and it has been reported to react ongoing inflammation and/or tissue damage⁵. CRP is phylogenetically a highly conserved plasma protein, with homolog in vertebrates and many invertebrates that participates in the systemic response to inflammation. Its plasma concentration increases during inflammatory states, a character that has long been employed for clinical purposes⁶. It was stated that CRP was increased after estrogen administration and that tissue remodeling and effective implantation require inflammatory microenvironment (that accompanied by increased chemokines, cytokines and their receptors) in the first trimester of pregnancy. On other hand too much inflammatory reaction may lead to recurrent abortion, further pregnancy complications like preterm labour or pre-eclampsia⁷.

One of the immunologic factors proposed for infertility is presence of antisperm antibodies in serum. Antisperm antibodies are found in cervical mucus, seminal plasma and sera of men and women⁸. It is possible for couples with antisperm antibodies occasionally to overcome the situation and become pregnant. Acquisition of genital tract infection following the initial pregnancy can lead to subsequent development of antisperm antibodies⁹.

Patients and Methods:-

Fifty eight women undergoing ICSI program treatment with an age 21 to 41years old were included in this study which was performed in High Institute of Infertility Diagnosis and ART/ Al-Nahrain University/ Baghdad/ Iraq and Kamal Al-Samarrai hospital for infertility treatment Baghdad/ Iraq. Eighteen women (31%) achieved pregnancy and was defined as pregnant group, while forty women (69%) failed to achieve pregnancy and was defined as non-pregnant group. C. reactive protein and antisperm antibodies were measured in serum and follicular fluid - and linked to oocyte and embryo quality.

Intracytoplasmic Sperm Injection procedure:-

Controlled ovarian stimulation (COS): All patients included in this study were subjected to short agonist ovarian stimulation protocol in which women received mid- luteal protocol down-regulation with GnRH agonist, triptorelin (Decapeptyl 0.1 mg, Ferring Co, Kiel, Germany)® by every day subcutaneous injection and the pituitary desensitization was completed by reaching the level of E2 < 50 pg/ml and endometrial thickness was ≤ 4 mm on ultrasound examination, the women received rFSH (Gonal F, Merck Serono)® containing 75 IU of FSH activity per ampoule by daily subcutaneous injection (2-5) ampoules according to age, weight and patient's response. Transvaginal ultrasound TVU was performed on cycle day 7 and subsequent scan was done every 2-3 days as required. The doses of (Gonal F) was monitored by follicle growth using TVU and serial serum E2 level until the day of hCG administration when ovulation was induced by injection of rhCG (Ovitrelle 6500 IU; Merck Serono) subcutaneously when at least (3-4) follicles ≥17mm in diameter were detected by ultrasound examination.

Collection and preparation of the oocytes and Intracytoplasmic Sperm Injection:-

Oocyte retrieval was performed by single lumen aspiration needle under vaginal ultrasound-guide 34-36 hours after hCG injection by the gynecologist under general anesthesia. All patients received progesterone every day starting from the day of the ova pick up (OPU). Under the inverted microscope at 100× the oocytes are scored as immature (prophase I), intermediate (metaphase I), mature (metaphase II), and atretic. Thereafter, the oocytes are incubated in CO₂ incubator for 30 minutes-1 hour. Immediately prior to micromanipulation, each oocyte is examined by the embryologist in IVF laboratory under the microscope to assess the maturation stage, metaphase II (MII) being confirmed by the absence of the germinal vesicle and the presence of two polar bodies. In (1 - 3) days after

injection checking of the embryo quality, under the inverted microscope (ICSI microscope) is done which shows 2 cell stage embryo, after 48 hr, 4-8 cell stage in subsequent days. Embryo transfer is performed only in embryos that have reached this level of maturity.

Collection of serum and follicular fluid:-

Blood samples of the 58 women who were included in this study were collected, on day of OPU immediately before the procedure and left in plain tube. The blood was allowed to coagulate for 30 minutes and then centrifugation was done to separate the serum for 15 minutes at 3000 rpm. Follicular fluid was obtained from the first retrieved follicle to avoid contamination of blood and aspiration media which is used during aspiration and collected in special conical tubes. Care was taken to avoid blood-contaminated samples.

CRP and antisperm antibodies measurement in serum and follicular fluid:-

Serum and follicular fluid CRP concentrations were determined by agglutination and serial dilution method for positive result. While anti-sperm antibody (ASA) concentrations were determined by using Enzyme linked immunosorbent assay (ELISA). The results were summarized in table (1).

Assessment of embryo quality: embryos were graded according to the following criteria:-

- *grade I: Equal size blastomeres with no cytoplasmic fragmentation
- *grade II: Equal blastomeres with less than 25% fragmentation
- *Grade III: Unequal blastomeres with no cytoplasmic fragments
- *Grade IV: Unequal blastomeres with minor cytoplasmic fragmentation
- *Grade V: Equal or unequal blastomeres with major cytoplasmic fragmentation
- *Grade VI: Few or no blastomeres with major cytoplasmic fragmentation⁽¹⁰⁾

Statistical analysis:-

Data were summarized, presented and analyzed using statistical package for social science (spss) and Microsoft office Excel 2007. Numeric variables were expressed as mean \pm standard error (SE), while nominal variables were expressed as number and percentage. Independent sample student Paired t-test was used to compare mean between two groups.

Results:-

- Serum CRP level: The mean \pm SE of Serum CRP level in all patients included in this study was (0.35 \pm 0.05) mg/ml. For group (1), it was (0.08 \pm 0.01) mg/ml, while in group (2) the mean \pm SE was (0.48 \pm 0.07) mg/ml. The statistical analysis showed highly significant ($P < 0.001$) difference in CRP level between the pregnant and non-pregnant groups as shown in figure (1).

- Follicular Fluid CRP level: The mean \pm SE of follicular fluid CRP level in all patients present in this study was (0.29 \pm 0.05) mg/ml. For group (1), it was (0.07 \pm 0.01) mg/mL, while in group (2) the mean \pm SE was (0.30 \pm 0.07) mg/ml (figure 2). The statistical analysis showed highly significant ($P < 0.001$) difference between the pregnant and non-pregnant groups.

- Serum ASA level: The mean \pm SE of serum ASA in all patients included in this study was (77.1 \pm 3.9) IU. For group (1), it was (62.6 \pm 3.1) IU, while in group (2) the mean \pm SE was (83.7 \pm 5.2) IU. The statistical analysis showed significant ($P < 0.05$) difference between the pregnant and non-pregnant groups as shown in figure (3).

- Follicular Fluid ASA level: The mean \pm SE of follicular fluid ASA in all patients present in this study was (73.5 \pm 4.1) IU. For group (1), it was (55.3 \pm 2.6) IU, while in group (2) the mean \pm SE was (81.7 \pm 5.3) IU. The statistical analysis showed highly significant ($P < 0.001$) difference between the pregnant and non-pregnant groups (figure 4).

Table (1):- Comparison of serum & follicular fluid CRP and ASA levels between pregnant and non-pregnant groups (Mean \pm SE)

parameters	Non Pregnant (n= 40) (Mean \pm SE)	Pregnant (n = 18) (Mean \pm SE)
Serum CRP (mg/ml)	0.48 \pm 0.07	0.08 \pm 0.017**
FF CRP (mg/ml)	0.3 \pm 0.07	0.07 \pm 0.01**
Serum ASA (IU)	83.7 \pm 5.2	62.6 \pm 3.1*

FF ASA (IU)	81.7 \pm 5.3	55.3 \pm 2.6**
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* Significant difference $p < 0.05$

** Highly significant difference $p < 0.001$

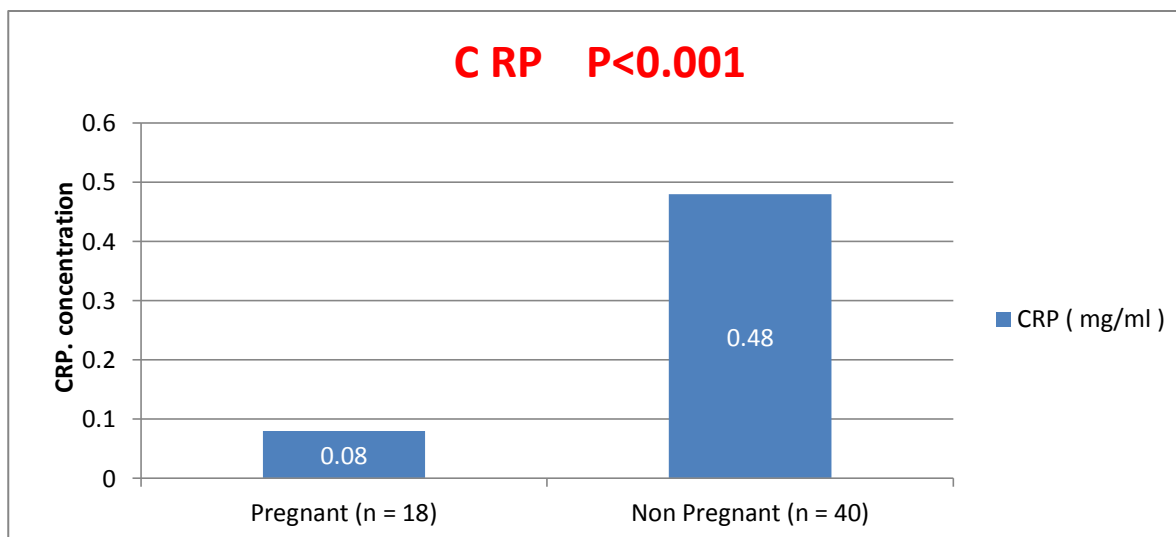


Figure (1): Comparison of mean Serum CRP levels between pregnant and non-pregnant groups.

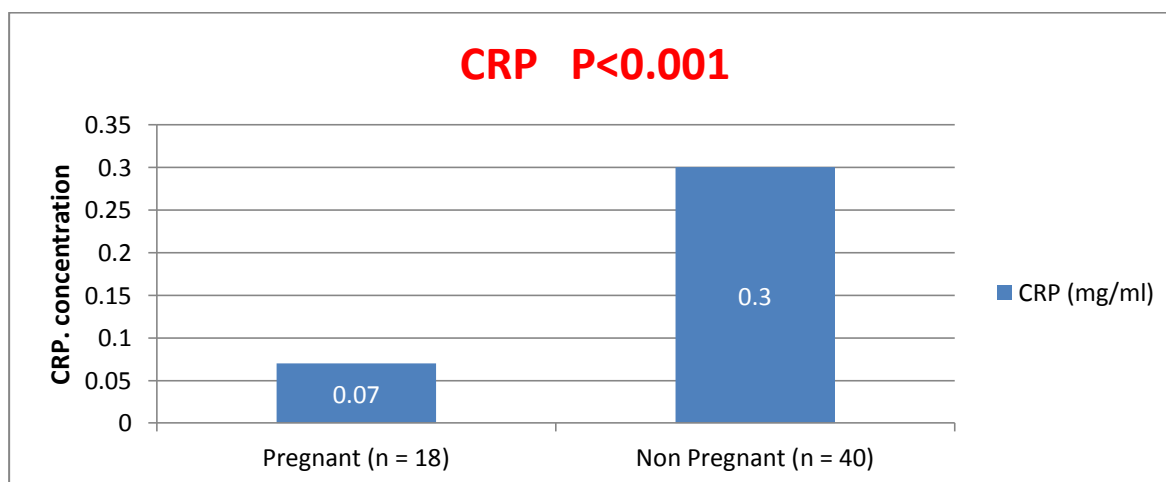


Figure (2):- Comparison of mean follicular fluid. CRP levels between pregnant and non-pregnant groups.

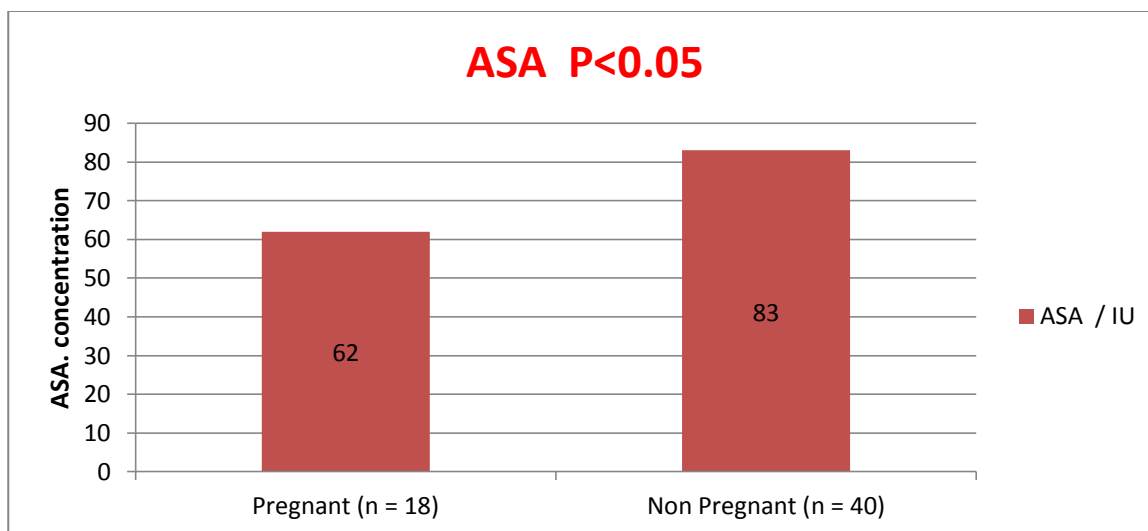


Figure (3):- Comparison of mean Serum ASA levels between pregnant and non-pregnant groups.

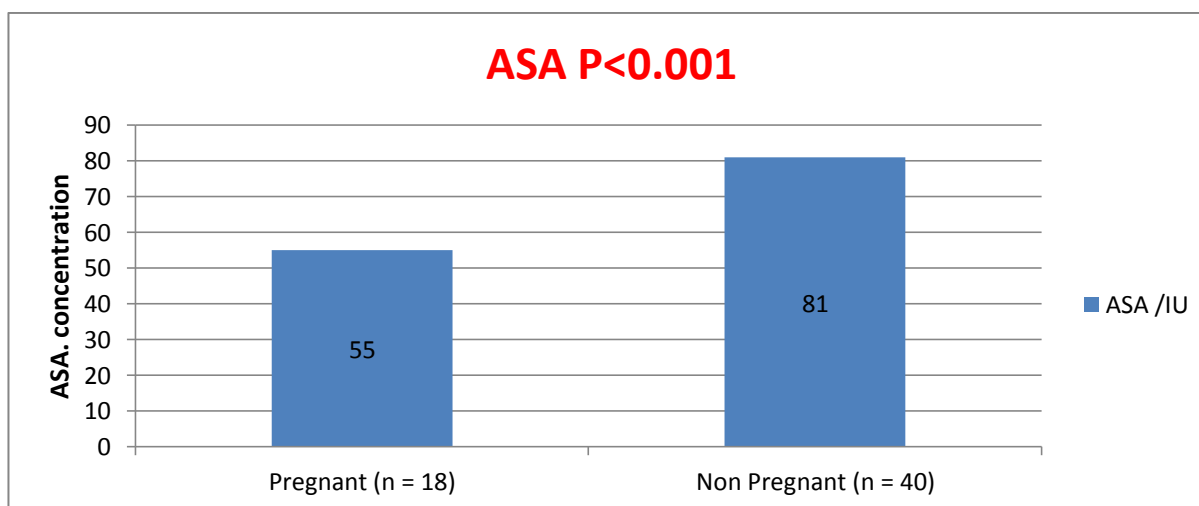


Figure (4):- Comparison of mean follicular fluid ASA levels between pregnant and non-pregnant groups.

Correlation of serum (CRP) and (ASA) levels and other clinical and cycle parameters in all infertile females as shown in Table (2).

Table (2):- Correlation of Follicular Fluid (CRP) and (ASA) levels with other clinical and cycle parameters in all studied females.

Correlation		M II	Grade I embryo	Fertilization Rate
S . CRP	(r)	-0.334 *	- 0.338 *	-0.382 **
	P-value	0.010	0.023	0.003
S . ASA	(r)	- 0.306 *	- 0.358 *	-0.438 **
	P-value	0.019	0.031	0.001
FF .CRP	(r)	- 0.268 *	- 0.267 *	- 0.403 **
	P-value	0.042	0.029	0.002
FF . ASA	(r)	- 0.315 *	- 0.348 *	-0.300 *
	P-value	0.016	0.044	0.022

* Correlation is significant.

** . Correlation is highly significant.

r Pearson Correlation

Discussion:-

Comparison of serum and follicular fluid CRP Levels between pregnant and non pregnant groups:

In Table (1), a significant difference was found in mean serum & follicular fluid CRP levels ($P < 0.05$) between the pregnant and non-pregnant groups. These results agree with a study done by Vakkx *et al* (2012) who found a strong correlation exists between serum and follicular fluid C-reactive protein (CRP) levels in women undergoing ART and pregnancy rate⁷. Elevated follicular fluid and serum CRP concentrations have found previously to be associated with chronic anovulation and non conception in women undergoing fertility treatment^{8,9}. High CRP levels are characteristic for increased inflammation and because the oocyte is in close contact with FF, an association is believed to exist between the content of FF and oocyte quality¹¹.

Also in this study a significant difference was noticed in mean serum and follicular fluid ASA ($P < 0.05$) between the pregnant and non-pregnant groups. These results in agree with studies done by Ackerman *et al* (1984) and Clarke *et al* (1985) who stated that women undergoing IVF-ET may be affected by the presence of antisperm antibody in their serum and FF leading to decrease oocyte quality and fertilization rate so it may significantly influence on success rates of the assisted reproductive techniques used to achieve conception^{12,13}. There is recent evidence that the fertilized egg shares some of the same antigens that are found on the sperm. It is possible that sperm antibodies present in the mother can react with the early embryo, resulting in its destruction^{or} may be due to inflammation in the oocyte environment inside follicle¹⁴.

Correlation of Follicular Fluid (CRP) and (ASA) levels and other clinical and cycle parameters in all infertile females

In Table (2) there was a significant negative correlation between the CRP and MII oocyte (which is mature oocyte), grade I embryo and also on fertilization rate, which mean that with increase in CRP, there is a decrease in oocyte quality and embryo quality. This result was similar to that of Soheila *et al* (2010) who stated that with increasing serum CRP level in day of embryo transfer rather than ovum pick up can predict the success in patients undergoing IVF/ICSI¹⁵. In women undergoing IVF the concentrations of CRP in blood increase significantly during the first week following oocyte retrieval. Successful outcome is associated with a relative small increment in CRP¹⁶ in addition to its effect on the quality and maturity degree of oocyte. Therefore FF content of CRP is believed to be related to fertilization, embryo development and implantation rate¹⁷.

Also in this study table (2) showed a significant negative correlation between the ASA and MII oocyte, grade I embryo and on fertilization rate, which mean that with increase in ASA, there is a decrease in oocyte quality and embryo quality. This result was in agreement with previous studies which reported that women with antisperm antibodies have several problems to overcome in order to achieve successful IVF-ET, such as a low fertilization rate and poor quality of transferred embryos¹⁸, so cumulus/oocyte complex washing and/or enzymatic cumulus removal are considered as elective interventions in the case of antisperm immunity. Each patient entering an IVF-ET program should have the antisperm antibody assay performed as a preliminary screening¹⁹. However, in another study a high implantation rate was observed in those with elevated ASA, even in women at advanced age. The occurrence of a cellular or humoral immune reaction against sperm may augment the uterine receptivity for the implantation of fertilized ova or blastocyst²⁰.

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