

**How to Cite:**

Taqi, S. N., & Al-Murshidi, S. Y. (2022). The correlation between the levels of anti-sperm antibodies and some cytokines in the seminal plasma with sperm parameters and leukocytes concentration of infertile men. *International Journal of Health Sciences*, 6(S10), 1145–1151. <https://doi.org/10.53730/ijhs.v6nS10.13845>

# **The correlation between the levels of anti-sperm antibodies and some cytokines in the seminal plasma with sperm parameters and leukocytes concentration of infertile men**

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**Abstract**---Background: The aim of this work is study the relationship between sperm parameters, anti-sperm antibody and some cytokines levels for infertile men. Semen samples were studied for 88 infertile men; of these (28) infertile men with normozoospermic, (29) with oligozoospermic and (31) with asthenozoospermic, samples were collected from Fertility Centre in Al-Sadder teaching hospital in Al-Najaf / Iraq during the period of (2/11/ 2021) and (5/2/2022). All participants underwent seminal analysis and measurement of seminal plasma antisperm antibody and some cytokine. The result of the study showed negative correlation between antisperm antibody and of interleukin (IL-1 and IL-6) with sperm parameter and positive correlation with leucocytes. The presence of leucocytes in the semen is associated with an increase in levels of interleukin (IL-1 and IL-6) in the seminal plasma of infertile men. The presence of antisperm antibody and interleukin (IL-1 and IL-6) in the seminal plasma is associated with a negative relationship in the semen quality of infertile men.

**Keywords**---anti-sperm antibodies, cytokines, seminal plasma, leukocytes concentration, infertile men.

## **Introduction**

Infertility is the inability to conceive after 12 months of regular, unprotected sexual activity due to a disturbance in one partner's reproductive capacity (Zegers-Hochschild *et al.*, 2017) 10-15% of couples have infertility; approximately 50% of the reasons of infertility are due to male issues such as insufficient sperm

count (oligospermia), low sperm motility, or defective sperm morphology (Shukla, et al.,2013). In 70% of cases, there are identifiable causes for male infertility, such as anatomical, immunological, physical or obstructive diseases, hormonal and environmental variables. However, in 30% of cases, there is no clear explanation for male infertility, which is referred to as idiopathic factors (A Rivera-Diaz, et al.,2021).

The anti-sperm antibodies (ASA) are one of the reasons of infertility, and the resulting infertility is known as immunologic infertility (Ashrafizadeh and Ahmadi.,2018). It is believed to be the cause of infertility in 10–30% of infertile couples and 12–14% of men (Cui et al.,2015). The ASA exerts its effects by altering the motility and agglutination of sperm (Tomlinson et al.,2013). In the male genital tract, the testes release cytokines that regulate the proliferation and differentiation of germ cells and mesenchymal cells and play a role in steroid anabolism (Jiang, et al.,2016). Several cytokines in seminal plasma, including as interleukin-1 (IL-1) and interleukin-6 (IL-6), have been associated with sperm concentration, motility, viability, and morphology (Jiang, et al.,2016; Aghazarian, et al.,2013).

### **Aim of the work**

study of the relationship between semen quality, anti-sperm antibody, some cytokines and zinc levels for infertile men.

### **Participants and methods**

The present study was conducted in the infertility center of Al-Sader Medical city in Al-Najaf governorate and in the laboratory of advanced research of the Department of Laboratory Investigations/ Faculty of Science/ University of Kufa. where carried out between (2/11/ 2021) and (5/2/ 2022) the study included a total of eighty-eight (88) semen samples where patients are divided to normozoospermic (28), oligozoospermic (29) and asthenozoospermic (23). The correlation between semen parameters and levels of antisperm antibody, some cytokine evaluated for all participants

### **Laboratory evaluation**

#### **Semen collection and evaluation**

Semen samples were collected from patients after 2–7 days of abstinence, the semen samples were examined after they were left in the incubator at 37°C to liquefy completely for about 30-60 minutes. After completing the liquefaction and removed abnormal viscosity the parameters of semen and sperm were examined microscopically and macroscopically. These parameters include: sperm concentration, sperm motility percent, progressive motility, sperm morphology percent and leukocytes, then the sample was centrifuged at 3000 (rpm) for 10 minutes to obtain the plasma. The seminal plasma was frozen in –20 Co for examination of antisperm antibodies and cytokine levels. The male partners were examined and the reproductive aspect is evaluated by the semen analysis according to the world health organization WHO (2010)

### **Semen Leukocytes evaluation**

Leukocytes were counted using the Leucoscreen method, Briefly, one drop of semen was mixed with one drop of working solution (Leucoscreen stain and hydrogen peroxide), covered with a cover slip for 2 min, and then the result was read at a magnification of  $\times 400$ . Peroxidase-positive cells stained yellow to brown, whereas other cells stained pink (Mahran, and Saleh., 2014).

### **Calculate the proportion of peroxidase-positive cells as follows:**

Proportion positive round cells  
Number of positive round cells

$$\frac{\text{(Number of positive round cells + Number of negative round cells)}}{\text{Total number of round cells}}$$

then calculate the concentration of peroxidase-positive white blood cells in the semen sample as follows:

concentration (mill/mL) = Proportion positive round cells x total concentration of round cells

### **Biochemical analysis**

After completing examination, the sample then was placed in conical test tube and centrifuged at 3000 (rpm) for 10 minutes to obtain the plasma. The seminal plasma was stored in Eppendorf tubes for frozen in  $-20^{\circ}\text{C}$  for examination of antisperm antibodies (ASA) and cytokine. Seminal plasma ASA and cytokine (IL-1 and IL-6) had been measured by the immunological method (Enzyme-Linked-Immuno-Sorbent- Assay) employs the quantitative sandwich enzyme immunoassay technique.

### **Statistical analysis**

Data of the current study was tabulated in Excel Microsoft Office Word software 2011, then transferred into computerized data form using two statistical software, the statistical package for social sciences (SPSS, version 23) and Medcalc Version 12.5 Correlations were conducted by using Pearson correlation Coefficient. P values  $\leq 0.05$  and  $\leq 0.01$  considered as significant.

### **Results and Discussion**

#### **Correlation between antisperm antibody levels in Seminal Plasma and Seminal Parameters for Infertile Men**

The result of the study showed negative correlation between antisperm antibody with sperm concentration, Progressive motility, normal sperm morphology at ( $p < 0.05$ ) but the results were revealed positive correlation between antisperm antibody with leucocyte count at ( $p < 0.05$ ). This result may be caused by the disruption of blood-testis barrier and this result is abnormal spermatogenesis. Our results agreed with the study that show in patient with ASA positive

infertility male that have reducing in sperm parameter while the patient with ASA negative controls that have normal sperm parameter, the ASA that stick on the surface of the sperm induces sperm cytotoxicity, increase sperm phagocytosis, so causing the decrease of the sperm concentration, (Cui, *et al*, 20 15)

ASA may decrease the motility of spermatozoa through agglutination and immobilization or interfering with sperm mucus interaction, thereby inhibiting sperm migration through the female genetic tract (Nagaria, *et al.*,2011). Both infection and sperm antigen sensitization can trigger the immune system to the formation of leucocytes, thereby forming ASA. Inflammation may lead potentially to genital tract disruption and ASA formation, Solis *et al.* (2003) observed that the presence of "anti spermatic" antibodies is associated to macrophage concentration, Al-Segar (2009) indicated that infections in the genital tract were correlated with the production of ASA. Some studies indicated a significant relationship between leucocytospermia and incidence of ASA (Salih *et al.*,2009; Alam and Ataurrahman, 2012) these results were agreed with current study

Table 1  
Correlation between antisperm antibody in seminal plasma and semen parameters of infertile men N= 88

Semen parameters	R=	p. value
Sperm concentration (Million/ml)	-0.192ns	0.073
Progressive motility (%)	-0.049ns	0.652
Normal sperm morphology (%)	-0.068ns	0.528
Leucocyte (Million/ml)	0.154ns	0.153

ns= Non significant correlation by using Pearson correlation coefficient

### **Correlation between Interleukin1B levels in seminal Plasma and Semen Parameters for Infertile Men**

As listed in table (3-2) there was high significant positive correlation. ( $p < 0.01$ ) between the concentration of seminal plasma Interleukin 1B in infertile men, and seminal leucocyte count ( $r = 0.406$ ). The table also showed that there was nonsignificant negative correlation between the concentration of seminal plasma Interleukin 1B in infertile men, whit each of sperm concentration ( $r = -0.204$ ) progressive motility ( $r = -0.118$ ) normal sperm morphology ( $r = -0.200$ ). Cytokines IL-1a, IL-1b, and TNF-a stimulate reactive oxygen species (ROS) production by fertile donor sperm at levels that are consistent with the levels of IL-1 occurring in human seminal plasma. ROS production has been demonstrated to impair sperm function. ROS-related sperm membrane peroxidation may be one mechanism by which cytokines can exert a detrimental effect on male fertility (Buch, *et al.*,1994)

Table 2  
Correlation between Interleukin 1B in seminal plasma and semen parameters of infertile men N=88

Semen parameters	R	P. value
Sperm concentration (Million/ml)	-0.204ns	0.055
Progressive motility (%)	-0.118ns	0.273
Normal sperm morphology (%)	-0.200ns	0.061
Leucocyte (Million/ml)	0.406**	0.000

\*\*=significant at  $P \leq 0.01$ , ns= Non significant correlation by using Pearson correlation coefficient

### Correlation between Interleukin6 levels in seminal Plasma and Semen Parameters for Infertile Men

The results reveal a high significant positive correlation at ( $p < 0.01$ ) between the concentration of seminal plasma Interleukin 6 in infertile men, whit Leucocyte count ( $r = - 0.273$ ). Also the results showed that there was nonsignificant negative correlation between the concentration of seminal plasma Interleukin 6 whit each of sperm concentration ( $r = -0.043$ ) progressive motility ( $r = -0.092$ ) and normal sperm morphology ( $r = - 0.143$ ). This study also agree with the study that showed levels of IL-6 in seminal plasma correlated positively with leukocyte count and negatively with sperm count, motility and morphology (Naz, and Kaplan., 1994).

Another study by Eggert-Kruse, *et al* (2001) was found no marked association between IL-6 and sperm count, morphology and A strong correlation of the seminal plasma IL-6 concentration with the leukocyte ratio. infection of male genital tract or increase the oxidative stress lead to IL-6 increase level (Fraczek, and Kurpisz., 2007.). In addition to inflammation of any part of reproduction specially prostatitis (Alshahrani, *et al.*, 2013).so that the natural of interleukin-6 function in spermatogenesis regulation and pro-inflammation effected by the failure of the sperm production process (Huleihel, and Lunenfeld., 2004).

Table 3  
Correlation between Interleukin 6 in seminal plasma and semen parameters of infertile men N=88

Semen parameters	R=	P. value
Sperm concentration (Million/ml)	-0.043ns	0.684
progressive motility (%)	-0.092ns	0.403
Normal sperm morphology (%)	-0.143ns	0.184
Leucocyte (Million/ml)	0.396**	0.000

\*\*=significant at  $P \leq 0.01$  ns= Non significant correlation by using Pearson correlation coefficient

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