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Original Research Article

The Role of Anti-Sperm Antibodies in Primary, Secondary, Immunological and Idiopathic Infertility in Men

Furat Latif Karim[1], Muneef Saab Ahmed[1*], Mazen Anwar Al-Obeidi[2]
[1]University of Tikrit, College of Education for Pure Sciences, Iraq
[2]University of Tikrit, College of Medicine, Iraq

***Corresponding Author:** Muneef Saab Ahmed
University of Tikrit, College of Education for Pure Sciences, Iraq

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Abstract: The current study aimed to conduct a statistical study to find out the numbers of men with primary and secondary infertility, immunological infertility and Idiopathic infertility in the city of Tikrit with an estimate of the levels of some sex hormones and physiological and immunological variables. The study included 400 married men who attended the Urology Department at Salah Al-Din Hospital and private clinics in the city of Tikrit and its environs for the period from April 2021 to February 2022, and their ages ranged from (20-50) years, and they were divided into two main groups as follows: It included (80) men, and it was divided into four sub-groups, namely: - The group of patients with primary infertility and included 20 individuals. The group of patients with secondary infertility included 20 individuals. And the group of immunocompromised patients included 20 individuals. And the group of patients with unexplained infertility included 20 individuals. The group of natural (fertile) individuals: It is the fifth group and represents the control group, which included (20) individuals. The results showed that the percentage of primary infertility was the highest among infertile patients, reaching 41%, while the percentage was lowest for immunological infertility, reaching 7%. While the percentage of secondary and unexplained infertility was 21% and 31%, respectively. On the other hand, the results showed that there were significant differences ($P<0.05$) in sperm parameters between the study groups, as sperm concentrations and the percentage of mobile sperm showed a significant decrease ($P<0.05$), especially in the secondary infertility group compared to the control group. As for the percentage of slow-moving sperms, there were no significant changes ($P<0.05$) in all infertile groups compared with the control group. As for the percentage of non-motile sperms, it showed a significant increase ($P<0.05$), especially in the unexplained infertility group compared with the control group. With regard to sperm shape, the percentage of deformed sperm showed a significant increase ($P<0.05$), especially in the primary infertility group compared with the control group. The results of the current study also showed that there were significant differences ($P < 0.05$) in the concentrations of hormones between the study groups, as the concentrations of testosterone showed a significant decrease ($P < 0.05$), especially in the primary infertility group compared with the control group, while the least significant decrease was recorded ($P<0.05$) in testosterone concentration in the unexplained infertility group. Regarding the prolactin hormone, a significant increase ($P<0.05$) was recorded in the infertility groups compared with the control group, as it was significantly higher in the secondary infertility group. LH also showed significant differences ($P<0.05$) between the study groups, and the highest significant increase was in the primary infertility group compared to the control group, while the unexplained infertility group did not show any significant differences compared to the control group. As for the FSH hormone, it showed significant differences ($P<0.05$) between the study groups, as it recorded the highest significant increase in the primary infertility group compared to the control group, while the unexplained infertility group did not show any significant differences compared to the control group. The results of the current study also showed that there were significant differences ($P<0.05$) in the concentrations of immunoglobulins between the study groups, as the concentrations of IgA showed a significant decrease ($P<0.05$), especially in the secondary infertility group compared with the control group, while the least significant increase was recorded ($P<0.05$). 0.05) in the concentration of IgA in the immunocompromised group. Regarding IgG, a significant increase ($P<0.05$) was recorded in the infertile groups compared with the control group, if the highest significant increase was in the immunocompromised group. While the primary and secondary infertility group did not show any significant differences compared to the control group. IgM showed significant differences (P < 0.05) between the study groups, and the highest significant increase was in the immunocompromised infertility group compared with the control group, while the primary and secondary infertility groups did not show any significant differences compared with the control group. Also, ASA concentrations showed a significant increase (P<0.05), especially in the unexplained infertility group, while no significant change (P<0.05) was recorded in the ASA concentration in the primary infertility group. With regard to white blood cells, the percentage of white blood cells showed a significant increase (P<0.05), especially in the group of immunocompromised infertility, while the percentage of white blood cells was lowest in the group of primary infertility.

Keywords: Anti-sperm antibodies, infertility in men, primary, secondary, immunological and Idiopathic infertility.

1-INTRODUCTION

Infertility is defined as the inability to conceive after at least one year of marriage and regular intercourse between a man and a woman, and affects about 8-12% of couples worldwide. And 20-25% of infertility can be due to the male alone (WHO, 2021). Primary infertility is used to refer to couples who have never had children, and secondary infertility refers to couples who have had at least one pregnancy but are unable to achieve it again (Kazemijaliseh *et al*., 2015). But the most common diagnosis is unexplained idiopathic infertility, which accounts for about 60-70% of patients. Persons with unexplained infertility have all standard sperm counts intact and normal, as well as the examination of the genital tract is normal, and the wife's examinations are also normal (Saleh *et al*., 2003).

One of the causes of infertility is immune reactions (autoimmune) against sperm cells, as studies have shown that there are anti-sperm antibodies (ASA) in 8-21% of infertile men, while it is found in 19-2.1% In fertile men, therefore, this data confirms that not all anti-sperm antibodies are responsible for infertility (Krause, 2009). Recently, there has been a lot of discussion about the role of the immune system in fertility, as the immune system works in general. It can protect the body from foreign cells and bacteria (Rümke *et al*., 2008), but sometimes problems within the immune system prevent it from working properly. New tests are now available to identify these immunological factors in infertility (Flint *et al*., 2015).

2-MATERIALS AND METHODS

2-1 Collection of sample

Samples were collected through direct interviews using a questionnaire designed for this study, medical history was taken and a questionnaire examination was conducted that includes several questions asked to each patient studied These patients were admitted to the Urology Department at Salah Al-Din Hospital and private clinics for the period from April 2021 to February 2022, and the inclusion criteria were as follows:-

- Infertile males who have never had children after 12 months or more of marriage.
- Infertile males who have had at least one pregnancy but have not conceived a second time.
- Sterile males for immunological reasons, which in turn led to the non-occurrence of pregnancy.
- Sterile males who have all normal sperm parameters as well as intact genital tracts and all marriage requirements are intact.
- Healthy and fertile males, and the same standards were applied to them in the sample test.

2-2 Semen collection

The samples were collected using the masturbation method, after a period of abstinence of not less than three days, in a sterilized wine container with a wide mouth, after giving the patient the necessary instructions on how to take the sample without causing external contamination as much as possible. With each patient through a direct interview with them in a special information form for each person, which contains a set of questions, namely: the patient's name, age, occupation, residence, duration of marriage, period of infertility, and type of infertility (primary, secondary, immunological, or unexplained). And the number of children, the presence of other diseases related to the male reproductive system, taking some medications that may affect fertility, previous operations, the extent of the patient's dealings with radioactive and chemical materials, and the person's height and weight were taken. After that, clinical examinations were performed by the specialist doctor and various microscopic instruments were used, as well as semen plasma was separated using a centrifuge at a speed of (3000 cycles / min) for 15 minutes. A micropipette was placed in new plastic tubes and placed in sterile test tubes and kept under freezing to be used later for various hormonal and biochemical examinations (King and Witman, 1994).

2-3 Collection of blood samples

Draw (5) ml of venous blood Blood was drawn from the brachial vein of the patient after disinfecting the skin with 70% alcohol using a disposable sterile syringe and then transferred to a sterile tube, for each patient using a wine syringe and placed in a test tube free of any anticoagulant , and left for (15) minutes in the incubator at a temperature of

25 degrees, then using a centrifuge at a speed of (3000 cycles / minute) to separate the blood serum from the rest of its components (Eliasson, and biochemistry, 1971).

2-4 Seminal fluid analysis
2-4-1 Visual examinations
2-4-2 The Microscopic examination
2-5 Determination of ASA Concentration in Semen by ELIZA
2-6 Hormonal test
2-7 Statistical analysis

The data of all tests were statistically analyzed by means of a calculator and using the (SPSS) Statistical Package of Social Science program, as it depends on the t-test (T-test) at a probability level of $0.05 \leq p$ to identify the degree of significant differences between transaction rates.

3-RESULTS AND DISCUSSION

3-1 The percentage of infertility among infertile people according to the study groups

Figure (3-1), shows Percentages and numbers of types of infertility that were dealt with in the current study, where the results showed that the percentage of primary infertility was the highest among infertile patients, as the percentage reached 41%, while the lowest percentage was for immunological infertility, reaching 7%, while the percentage of infertility reached Secondary and unexplained 21% and 31%, respectively.

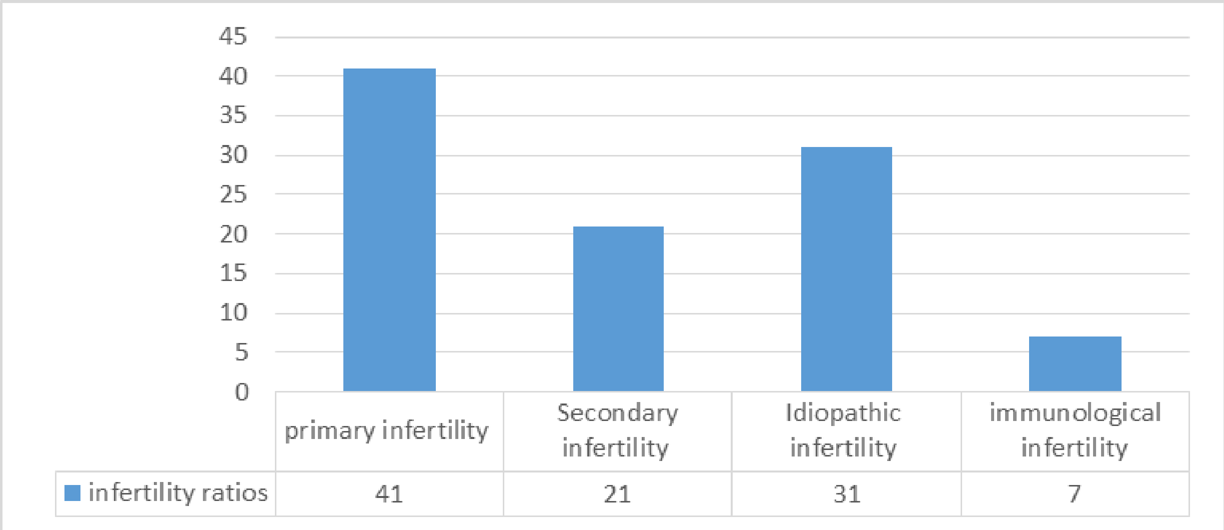

Figure (3-1): Infertility rates in the study groups (%)

3-2 Sperm Standards

The results of the current study, as shown in Figure (3-2) showed that there were significant differences (P<0.05) in the sperm parameters between the study groups, as the sperm concentrations showed a significant decrease, especially in the secondary infertility group, as the sperm concentration reached (2.75 ± 33.97)10^6 / ml compared to the control group (7.48 ± 63.11) 10^6 / ml. With regard to sperm motility, the results of the current study showed in Figure (3-3) that the percentage of moving sperm showed a significant decrease, especially in the secondary infertility group, as it reached Sperm motility (3.59 ± 8.02)% compared to the control group (17.51 ± 62.83)%. As for the percentage of slow-moving sperms, as shown in Figure (3-4), there were no significant changes in all infertile groups compared with the control group (6.05 ± 25.33). As for the percentage of non-motile sperm in Figure (3-5), it showed a significant increase (P<0.05), especially in the unexplained infertility group, as the percentage of non-motile sperm reached (5.41 ± 54.13)% compared to the control group (4.57 ± 13.91)%. With regard to the shape of sperm, the percentage of distorted sperm showed a significant increase, especially in the group of primary infertility, as the concentration of distorted sperm reached (6.81 ± 55.86)% compared to the control group (9.81 ± 15.26)% as shown in Table (3-1).

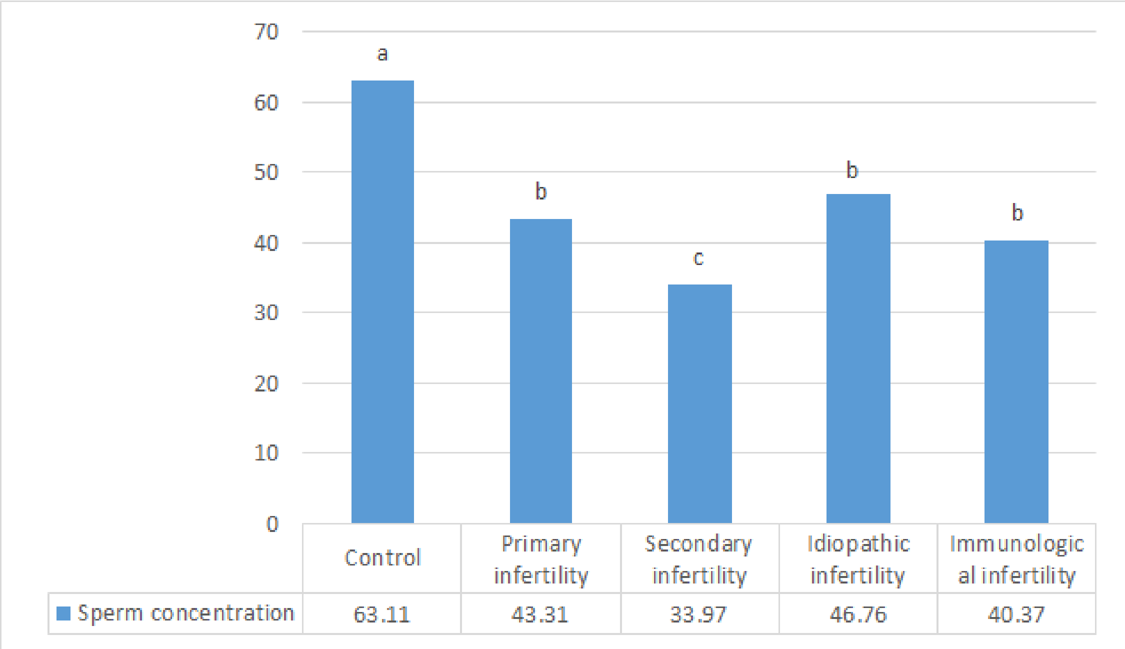

Figure (3-2): Sperm concentrations among the study groups (10^6 / ml)

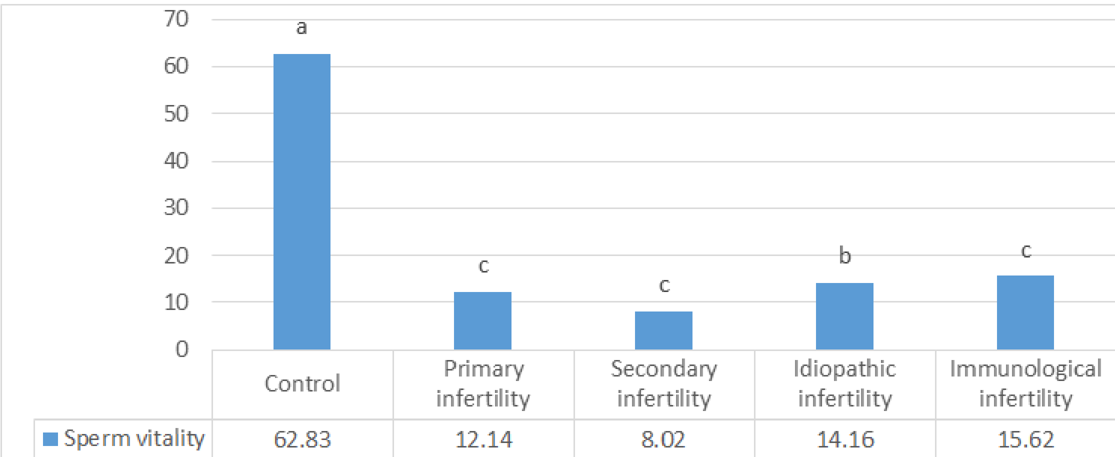

Figure (3-3): Percentages of sperm vitality among the study groups %

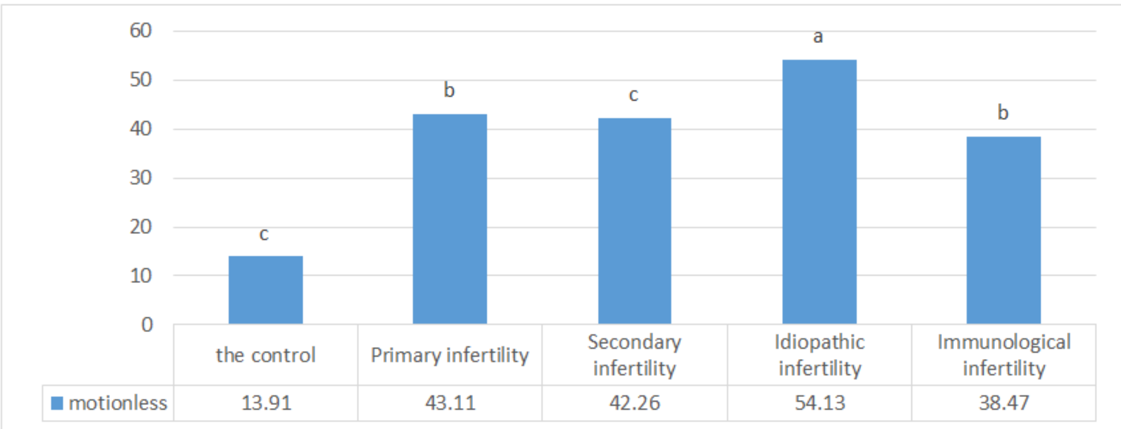

Figure (3-4): Percentage of immobile sperm among the study groups %

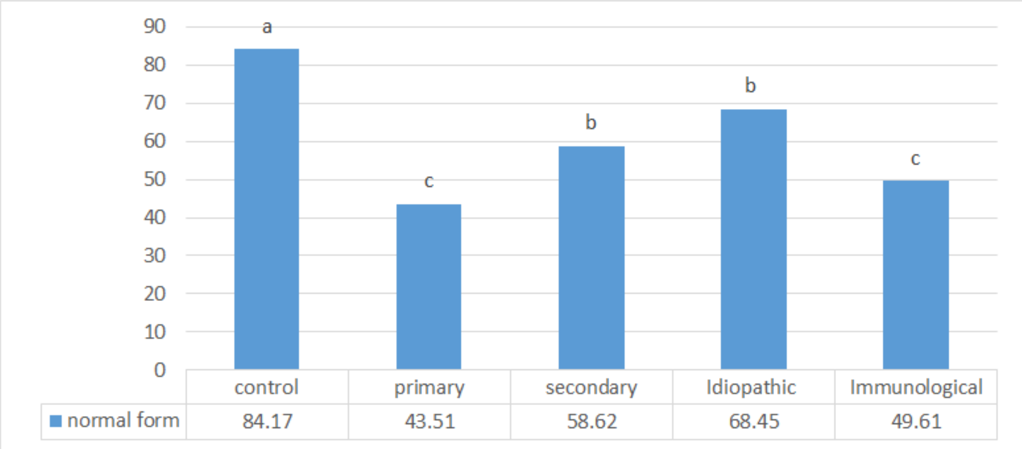

Figure (3-5): Percentages of normal-shaped sperm among the study groups %

Table (3-1): Percentages of some sperm parameters among the study groups (mean + SE)

Totals / Variables	Sperm concentration 10^6 /ml	movement % moving	Slow-moving	immobile	Sperm form% natural	distorted
control	7.48±63.11 a	17.51±62.83 a	6.05±25.33 a	4.57±13.91 c	7.21±84.17 a	9.81±15.26 d
Primary infertility	4.12±43.31 b	1.32±12.14 c	2.25±29.79 a	1.67±43.11 b	10.58±43.51 c	6.81±55.86 a
Secondary infertility	2.75±33.97 c	3.59±8.02 C	4.76±49.82 a	2.45±42.26 c	9.95±58.62 b	6.53±41.11 b
Idiopathic infertility	1.93±46.76 b	0.38±14.16 b	2.12±31.05 a	5.41±54.13 a	11.7±68.45 b	4.99±31.01 c
Immunological infertility	5.67±40.37 b	7.05±15.62 c	5.82±46.39 a	7.48±38.47 b	8.04±49.61 c	10.38±50.47 a

3-3 Levels of Sexual hormones

The results of the current study, as shown in Figure (3-7) showed the presence of significant differences (P<0.05) in the levels of hormones between the study groups, as the testosterone level showed a significant decrease (P<0.05), especially in the primary infertility group, as The testosterone level was (2.65 ± 1.002) ng/ml compared to the control group (9.115 ± 3.567) ng/ml, while the least significant decrease (P<0.05) was recorded in the testosterone level in the unexplained infertility group (3.715 ± 1.251) ng/ml. With regard to the prolactin hormone, as in Figure (4-10), a significant increase (P<0.05) was recorded in the infertility groups compared with the control group (8.07 ± 1.394), as the highest significant increase was in the secondary infertility group (21.16 ± 4.397) as shown in Figure (3-7).

The LH hormone level showed significant differences (P<0.05) between the study groups, including the highest significant increase in the primary infertility group (16.87 ± 3.971) ng/ml compared to the control group (4.79 ± 1.618), while the unexplained infertility group did not appear (7.72 ±). 2.15) ng/ml, any significant differences compared to the control group, as shown in Figure (3-7). As for the FSH hormone, it also showed significant differences (P<0.05) between the study groups, as it recorded the highest significant increase in the primary infertility group (9.43 ± 1.869) ng/ml compared to the control group (5.205 ± 1.644) ng/ml, while the group did not show Unexplained sterility (6.675 ± 2.534) ng/ml, no significant differences compared to the control group as shown in Table (3-2).

Table (3-2): Levels of sex hormones among the study groups (ng/ml)

Variables / Totals	Testo	Prolac	LH	FSH
control	9.115a±3.567	8.07c±1.394	4.79c±1.618	5.205d±1.644
Primary infertility	2.65c±1.002	19.39ab±6.23	16.87a±3.971	9.43a±1.869
Secondary infertility	3.393c±0.987	21.16a±4.397	13.748ab±3.767	7.905bc±1.878
Idiopathic infertility	3.715c±1.251	16.86b±6.11	7.72c±2.15	6.675cd±2.534
Immunological infertility	5.615b±1.098	11.33c±3.257	11.98b±8.45	8.56ab±2.867
P-Value	0.0008	0.0007	0.0001	0.0003

The current study found a significant difference in average serum testosterone levels among infertile men with significantly low testosterone level, and high levels of FSH in the presence of low testosterone levels are associated with primary hypogonadism, the level of testosterone in this study is low and lower than the control group This is consistent with the results of (Ramesh *et al*., 2004) who indicated a decrease in testosterone level in people with primary and secondary infertility, that the decrease in testosterone levels in primary infertility can be directly linked to the decrease in sperm parameters for this group, as it led to a rise in spermatogenesis, where it plays an essential role; Because it contributes to the initiation and maintenance of spermatogenesis, and this is consistent with the results of Di Guardo *et al*., 2020), while the results differ with (Hart *et al*., 2015), where they studied a large group (365) of infertile young men and did not find any relationship between sperm levels. Testosterone and semen standards.

In this study, the mean levels of prolactin, FSH, and LH in the blood were significantly higher in the infertile groups compared to the control group, while the average level of testosterone in the blood was lower in the infertile groups compared to the control group, and this result agreed with the results of (Gangwar *et al*., 2020), which indicated an increase in the level of prolactin, FSH, and LH in patients with primary and secondary infertility, on the contrary, no significant differences were found in the level of testosterone between primary and secondary infertility cases compared to the control group in a study ((Eniola *et al*., 2012). A significant increase in prolactin levels may result from the secretion of prolactin in a pituitary adenoma that produces galactorrhoea. The mechanism that leads to an increase in the level of prolactin in the blood to impaired spermatogenesis in normal men is not certain (Arowojolu *et al*., 2004), and elevated levels of prolactin in the blood have a detrimental effect on male reproduction by inhibiting the pulsatile release of gonadotrophins from the anterior pituitary gland. With a direct effect on spermatogenesis, treatment of established hyperprolactinemia with dopamine agonists results in significant improvements in both semen parameters and hormone levels (Dabbous and Stephen, 2017).

A personal view of male infertility based on statistical results and examination of sperm and hormone parameters, a condition that is not only extremely prevalent, but also a major reason for couples resorting to surgical treatment such as ICSI as a form of facilitated fertilization, and these procedures have a revolutionary effect on our ability to propose Treatments for male infertility cases associated with severely compromised semen quality, that the widespread use of these techniques poses risks in terms of miscarriage, the health and well-being of the offspring, and the persistence of the sterile phenotype in future generations, and discourages interest in male sterility or the development of non-surgical treatment strategies targeting the male patient, rather than of surgeries. As a result, although progress in elucidating the pathological mechanisms responsible for male infertility is very slow, genetic and epigenetic defects are certainly implicated in many cases, and may involve splicing mutations/defects affecting testicular RNA profile integrity, as well as overall motility for the copying process. In addition, spermatogenesis is disrupted by a variety of factors (age, smoking, obesity) many of which are believed to affect fertility and sperm DNA integrity by creating oxidative stress.

3-4 Estimation of the concentration of ASA in the semen of infertile men

The results of the current study, as shown in Figure (3-8), showed that there were significant differences ($P<0.05$) in the concentrations of sperm antibodies between the study groups. ASA (100.66 ± 33.99) u/ml compared to the control group (306.7 ± 36.93) u/ml, while no significant change ($P<0.05$) was recorded in the concentration of ASA in the primary infertility group (33.94 ± 17.02), as shown in the figure (3-8), with regard to white blood cells, the percentage of white blood cells showed a significant increase ($P<0.05$), especially in the group of immune infertility, as it reached (100.66 ± 33.99) 106 / ml, while the lowest percentage of white blood cells was in the group The initial sterility is (33.94±2.42) 10^6/ml, as shown in Figure (3-6).

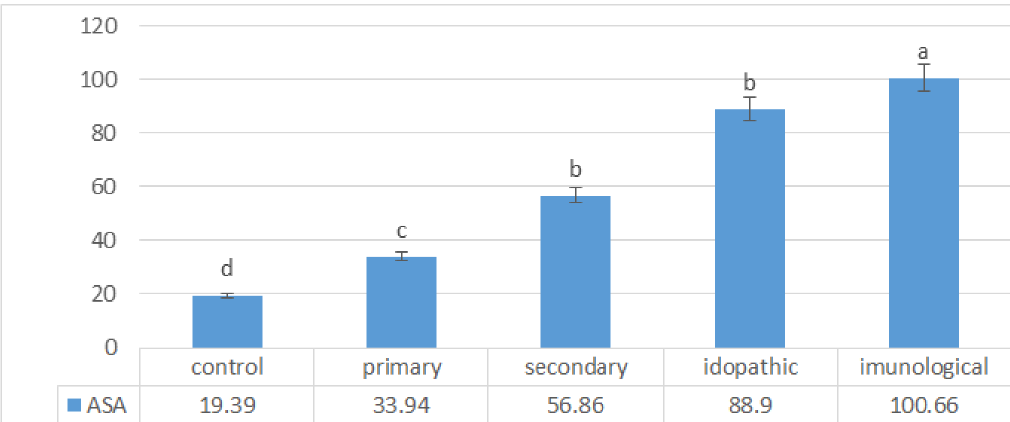

Figure (3-6): ASA concentrations in semen among the study groups (u/ml)

The results of the current study showed that there was a significant increase in the concentration of ASA among the infertile groups, especially in the immunocompromised group, and it is reported that ASA is also present in fertile men, but in lower concentrations for unknown reasons or the function is still not properly explored, this fact indicates That the mere presence of ASA does not directly disrupt fertility or the fertilization process, and there are several reasons that lead to high ASA in immune infertility, including: breakdown of the blood and testicular barrier, surgical trauma, various microbial infections, prostatitis, orchitis, testicular cancer, varicocele, and sex Unsafe, elevated ASA in the immunocompromised infertility group led to differences in sperm parameters, and recorded a significant increase compared to the control group. Evaluation of elevated ASA in semen accompanied by elevated immunoglobulins (IgA-IgG-igM) in immunocompromised infertility can be used as a diagnostic marker to predict obstruction of the vas deferens and epididymis. And the occurrence of infections in the male reproductive system, the current study agrees with a study (Cui *et al*., 2015) in terms of the effect of ASAs on different semen parameters, although ASA In males it was directly associated with male infertility, however, the overall effect of ASA on reproductive competence in females has not yet been explored, although reports indicate a lower incidence of spontaneous pregnancies (Simopoulou *et al*., 2019).

Several previous studies have demonstrated that ASA has a role in the pathogenesis of infertility (Sreenivas *et al*., 2011; Khan *et al*., 2012). However, this fact remains controversial, as other researchers have found no significant difference in ASA levels between infertile and normal men (Berek, 2007). The current study recorded a relatively high incidence of ASA in the seminal plasma among infertile patients of all kinds, and this result is very similar to the percentage of positive serial ASA (21.42%) recorded in the city of Mosul, where the drawer agglutination test (TAT) was used, and Shaya (AlDabbagh, 2012). This percentage is also very similar to the results presented by (Abdulla., 2009), who indicated that the percentage (20.1%) of semen was positive for ASA, a similar result obtained by TAT methods indicated that about (22.3%) of infertile males have a positive ASA result (Al-Khayat, 2004), and this result comes in conjunction with several previous studies that used different methods to detect seminal ASA (Al-Daghistani *et al*., 2010) conducted a study of 150 Infertile Jordanian men, indicating that about (25.34%) of them are positive for seminal ASA (Al-Daghistani *et al*., 2010), and (Abdulla. 2009) reported a slightly higher rate (29.94%), while a slightly lower rate was recorded (21.42%) in another study (Shaya and AlDabbagh, 2015), as well as the results of the current study are consistent with similar studies recorded A relatively similar percentage (32.1%) in the semen of men with unexplained infertility (Jabbar, 2011). The results of the current study indicated that ASA differences between infertile patients and normal subjects support the idea of using ASA assays as predictive tools for diagnosing infertility, as they can discriminate more among infertile and subfertile persons.

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