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The effect of some physiological variables on the semen of men recovered from COVID-19 in Fallujah city

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***Abstract*---**The current study aimed to know the effect of some physiological and hormonal variables in the blood serum of 60 samples of recovered men from COVID-19, their ages range from 20 to 49 years old, some of them were infected once and some of them were infected more than once, while the control group included 30 samples of healthy men, The samples were collected in Fallujah Teaching Hospital and from some private laboratories in the city of Fallujah. The sample collection period was from 10/15/2021 to 7/2/2022, during which semen samples were collected and the characteristics of semen were known, in addition to knowing the concentration of fructose sugar in semen

***Keywords*---**sperm concentration, total sperm, sperm motility, fructose in seminal**.**

Introduction

According to the reports of the World Health Organization (WHO) (WHO, 2021), viral diseases continue to emerge, and these diseases represent a serious health problem. The first time that human coronaviruses were identified was in 2002. These viruses cause many diseases, which can cause acute respiratory infection. The cause of this disease is SARS-CoV, a previously unrecognized coronavirus that is now facing The world is once again a serious health threat from COVID-19, caused by another new coronavirus (SARS-CoV-2), which is closely related to SARS-CoV. However, SARS-CoV-2 is more contagious and this strain has already infected a much higher proportion than previous strains and the virus has been shown to have differentiated effects on male reproductive health (Li *et al.*, 2020). It is therefore important to determine whether SARS-CoV-2 infection can affect

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male fertility as an immediate or long-term consequence of the disease (Dutta and Sengupta, 2021) because SARS-CoV-1 was able to affect the testicles (Khali *et al.*, 2020) It is known that some viruses such as HIV as well as mumps and viral hepatitis, can enter the testicle and cause viral orchitis, and in some cases may cause male infertility and testicular tumor (Xu *et al.*, 2006)

Infertility is defined as a disease characterized by the failure to conceive after approximately 12 months of regular sexual intercourse and is an important factor in the occurrence of pregnancy (Zegers-Hochschild *et al.*, 2017). That between 12% to 15% of sexually active couples suffer from infertility, and men contribute about 50% of cases in general at the present time (Machen and Sandlow, 2020). The most common causes of infertility are cases in which the patient suffers from low sperm count, oligospermia, which accounts for 35%, and the other case is asthenospermia, which accounts for 30% of infertility cases, in addition to the presence of other cases (Patki *et al.*, 2008).

Material and Methods

Semen Collection

Semen samples were collected by masturbation after abstaining from sex for 3-5 days and collected directly in a clean, sterile plastic container, after giving the patient the necessary instructions to prevent any contamination, the samples were taken from the patients and the name of the patient was written on the cup And transferred directly in a water bath at 37 ° C for 30 minutes to allow liquefaction, then the patient's information was taken and recorded in a special form for each person. Then, macroscopic and microscopic examinations of the semen were performed according to the standards and procedures recommended by the World Health Organization (WHO, 2010).

Macroscopic and microscopic examinations of the semen fluid

Macroscopic Examination

A. Color & Appearance
   The color and appearance were examined by direct macroscopic examination of the sample after the liquefaction. The normal sample is characterized by its bright gray color and homogeneous appearance. The sample may appear less opaque in the case of low sperm concentration or be red in the case of the presence of red blood cells in the sample or It is yellow in case the patient has jaundice. (WHO, 1999)
B. Volume
   The semen volume was measured using a sterile graduated cylinder. The volume of the semen sample is considered low-volume if the volume is less than 2 ml, and the volume is considered to be more than 6 ml over-volume. (WHO, 1999)
C. Liquefaction time
   The semen sample was liquefied within 20-30 minutes. The sample was gently mixed when placed in the incubator at a temperature of 37°C to reduce the liquefaction time. After that, the sample was examined to check

liquefaction and the liquefaction time was recorded when the entire sample turned into a liquid. (WHO, 2010)

D. Viscosity

The viscosity of the semen was estimated by gentle pulling into the micropipette. Then the semen is allowed to fall out of the pipette and the length of the suture is observed. The normal viscosity was determined when the sample could be poured as droplets. Abnormal viscosity will form a thread more than 2 cm long. If drops do not form and semen cannot be easily drawn into the pipette, this indicates a high viscosity. (WHO, 2010)

E. PH

The pH of the semen was measured by means of a graduated PH paper, the paper was dipped in the semen and by comparing the resulting color of the paper with the color corresponding to the standard tape, the pH value was determined. The pH is normal when it is alkaline between 7.2-8.0. (WHO , 2010)

Microscopic examinations:

A drop of the well-mixed semen sample was placed on a clean glass slide by means of a micropipette. The glass slide was covered with a slide cover and waited for one minute for the model to settle. Microscopic examination included estimation of potency, total number, differential sperm shape and number of round cells. (WHO, 2021)

A. Sperm Concentration

The sperm concentration was calculated by placing one drop (100 µl) of the semen sample on a glass slide and covered with a cover glass. This was done by calculating the average number of sperm in 10 random microscopic fields under the power of a magnification lens (40X). Number in Factor (106) (WHO, 1999)

Sperm concentration (million/ml) = number of sperm in HPF x 10^6

Sperm concentration is considered normal if it is equal to or greater than 20 x 10^6 sperm/ml

B. Sperm Count:-

The total sperm count was calculated by multiplying the sperm concentration by the total sample volume. (WHO, 1999)

Total sperm count (million/ejaculate) = sperm concentration x volume.

C. Sperm Motility:

Sperm motility was estimated by placing a specific volume (10µl) immediately after the end of the liquefaction time on a clean glass slide and placed in the incubator for 5 minutes at a temperature of 37 C°, covered with a slide and placed under a microscope with a force of 40X and the estimate was done at room temperature (20-27 C°) 100 sperm were counted in 10-5 microscopic fields selected randomly, and the percentage of sperm was recorded according to their movement (WHO, 1999)

Percentage of motile sperm = number of motile sperm / total number of sperm x 100

Sperm were classified into:-

(A) = Rapid progressive motility

(B) = slow or sluggish progressive motility

(C) = non progressive motility
(D) = Immotile
When a semen sample contains less than 40% of motile sperm, this is called asthenozoospermia

D. The morphology of spermatozoa
Morphologically normal sperm was examined by using the same slides prepared for sperm motility. At least 100 sperms were counted. The percentage of morphologically healthy sperm was using the following equation: Percentage of normal sperm = number of normal sperm / total number of sperm x 100 As for abnormal or abnormal sperms, at least 100 sperms are calculated in the sample, and then the percentage of abnormal or abnormal sperms is calculated using the following equation: Percentage of abnormal sperm = number of abnormal sperm / total number of sperm x 100 (WHO, 1992)

Measurement of fructose concentration in semen

Fructose in semen was measured through a special kit containing fructose solution from the Chinese company Biomarker. the result Changing the color to pink or red means that the result is positive, but if we do not notice a change in color, that means that the result is negative.

Statistical analysis

All study results were analyzed using the Statistical Package for the Social Sciences (SPSS) program. Using the Completely randomized design (CRD) system, the arithmetic mean was compared using the t-test to determine the variance between two groups of studied traits at the level of probability ($P \leq 0.05$) (Steel and Torey, 1980).

Results and Discussion

Semen specification

Semen Volume

The results of Table (1) showed that there was an insignificant decrease among the group of recovered patients compared with the control group at a significant level of $P \leq 0.05$. The results of this study agree with the results of the researcher (Guo *et al.*, 2021) when he studied 41 people who had been cured of COVID-19 and semen tests were conducted after about 56 days of infection, and this explains that the viscosity of the semen of the recovered people was not affected, The results are also consistent with (Kamil, 2021) in its results when studying the effect of COVID-19 on the recovered during the three months after infection.

Viscosity

The results from Table (1) showed that there was no significant difference between the group of those who recovered from COVID-19 and the control group at a significant level $P \leq 0.05$. The results of this study agree with (Kamil, 2021) in its results when studying it on a group of convalescents.

PH

The results of Table (1), showed that there was no significant difference in the pH value between the group who recovered from COVID-19 and the control group at a significant level of $P \leq 0.05$.

Sperm Concentration

The results showed from Table (1), that there was a significant decrease in sperm concentration in the group who recovered from COVID-19 compared to the control group at a significant level $P \leq 0.05$ These results are in agreement with the results of (Abdel-Moneim, 2021), (Guo *et al.*, 2021) and (Tiwari *et al.*, 2021), and the results can be interpreted that the emerging corona virus may affect the sperm concentration and its decrease during the infection stage through the effect of The virus affects the gonads responsible for the formation of a sufficient number of sperm, or through its effect on the Leydig and Sertoli cells in the testicle when it enters the cell through the enzyme converting angiotensin II. Also, the fever that the person was exposed to during infection, inflammation, or medications can affect the hypothalamic axis. Hypothalamus - pituitary gland - male gonads (Ma *et al.*, 2021)

Total sperm

The results of Table (1) showed a significant decrease between the study group compared with the control group at a significant level $P \leq 0.05$, which indicates that the infection of the Corona virus may be responsible for the weakness of the testicular function in the production of sperm or It may be the result of inflammatory responses or inflammation associated with fever, and these results are consistent with the findings of the researcher (Abdel-Moneim, 2021) and the researcher (Tiwari *et al.*, 2021). In a previous study conducted by the researcher (Yang *et al.*, 2020) revealed weakness in testicular tissue and a decrease in Leydig cells in infected persons, and thus these factors reflect a negative impact on the male reproductive system and thus negatively affect sperm formation.

Normal morphology

The results of Table (1) showed that there was a significant decrease in the recovering group compared to the control group at a significant level $P \leq 0.05$. The results of this study agree with (Mannur *et al.*, 2021), which explains that the formed sperms are deformed or of abnormal shapes, and therefore these sperms cannot perform their function to fertilize the egg

Abnormal morphology

The results of Table (1) showed that there was a significant increase in abnormal shapes in the group of recovered patients compared to the control group at a significant level $P \leq 0.05$. The results of this study agree with the results of

(Mannur *et al.*, 2021), which indicates the effect of the virus on the morphology of the sperm, which loses its main function in reproduction.

Motility

The results from Table (1) showed a significant decrease in the group of those who recovered from COVID-19 compared to the control group at a significant level of $P \leq 0.05$. The results of this study are in agreement with the results of the researcher (Tiwari *et al.*, 2021) in his study on a group of recovered patients, which showed a decrease in the progressive motility of sperm, which may be due to a decrease in the testicular lipid hormone as a result of infections associated with infection or as a result of fever that damages the germ cells. Or an increase in luteinizing hormone may lead to an imbalance in the gonads, as well as cytokines playing a role in inflammation of the seminal vesicles, prostate and epididymis (Suryasa et al., 2022).

Table (1) Semen characteristics of the Recovered group and the control group

SEMINAL FLUID			
Variable	Groups	No.	Mean± Std.
volume	Recovered	60	2.85± 1.16 N.S
	Control	30	3.43± 1.77
viscosity	Recovered	60	1.483± 0.368 N.S
	Control	30	1.483± 0.307
PH	Recovered	60	8.058± 0.208 N.S
	Control	30	8.083± 0.324
Concentration	Recovered	60	26.6± 4.60 *
	Control	30	42.5± 5.41
Total count	Recovered	60	74.9± 18.1 *
	Control	30	137.3± 27.6
Normal morphology	Recovered	60	60.5± 11.5 *
	Control	30	70.2± 11.9
Abnormal morphology	Recovered	60	70.2± 11.9 *
	Control	30	60.5± 11.5
Active motility	Recovered	60	17.02± 3.20 *
	Control	30	22.53± 3.50

The concentration of fructose in seminal plasma

The results showed after measuring the positive and negative fructose sugar in the semen of the recovering group from COVID-19 compared to the control group, and it showed an increase in the number of samples for fructose sugar in the semen of the recovered group, as the number of samples in the group of recovered patients in which the positive result appeared is 15 and the result is negative is 45 samples, As for the control group, the number of samples in which the positive result appeared is 2 samples, and the result is negative is 28, as shown in Table (2). Before the sperms as a result of a decrease in the number of sperms, which decreased in number as a result of COVID-19 infection, and this was confirmed by the study, as there is an inverse relationship between the concentration of fructose sugar in the semen and the number of sperms for the study group. These results are in agreement with the results of previous studies conducted by some researchers, including (AL-Samaaraie, 2013) and (Coppens, 1997), where their results indicated the presence of an increase in the concentration of fructose sugar for groups of people with oligospermia and asthenozoospermia compared to the control group that represents fertile people.

Table (2) of the concentration of fructose sugar in the Recovered group and the control group

Variable	Groups	Positive	Negative	Total
Fructose In Seminal	Recovered	15	45	60
	Control	2	28	30

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