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# The Relationship between the Sexual Abstinence Period and Sperm Parameters in Normozoospermia Infertile Men

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**Annotation: Background:** According to WHO guidelines, the abstinence period for semen analysis ranges from 2 to 7 days. The current study aimed to study the relationship between the abstinence period and semen parameters represented by the concentration of the sperm, the progressive movement of the sperm, the normal shape of the sperm, and the volume of semen in Normozoospermia patients. Methods: The study was conducted in the laboratories of the Fertility Center of Al-Sadr Teaching Hospital in Al-Najaf Governorate - Iraq. The age of the patients ranged from 20 to 44 and the marriage period ranged from 1 to 8 years. The results of the semen analysis of the samples were recorded and a group of normozoospermia was determined based on the World Health Organization's guideline. The relationship between the abstinence period and the parameters of semen represented by the sperm concentration, the progressive motility, the sperm normal morphology and the semen volume and leukocytes count were studied. **Results:** the results of our study showed found a

significant positive correlation between abstinence period with sperm concentration (r=0.24,p=0.015) while the results were revealed nonsignificant negative correlation between abstinence period with progressive motility and normal sperm morphology.

**Introduction:**

As male factors contribute to between 30% and 50% of cases of infertility1, 2, the examination of semen quality is a standard element in a male fertility assessment. Sperm quality not only in uences natural conception but also in uences the outcome of intrauterine inseminations (IUI) and in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI). Of particular importance are sperm concentration, the percentage of motile spermatozoa, and the percentage of morphologically normal spermatozoa3–4. The abstinence period prior to semen analysis or prior to providing sperm for fertility treatments currently recommended by the World Health Organization (WHO) is 2–7 days - 5. However, the manual gives no specie c background for the selection of this exact time period. The length of the abstinence period has been reported to have an effect on sperm concentration, motility, and morphology in normozoospermia men. However, the ideal abstinence period, especially in subfertile men, is a subject of controversy, e.g. abstinence time might in uence semen parameters differently in fertile and subfertile men [6,7]. While some studies show a positive effect of abstinence times shorter than 2 days on sperm concentration and motility in subfertile men [8, 9], other authors found an increased motility after an abstinence period of longer than 4 days[10,11], The magnitude of change in sperm parameter values after different abstinence periods can be considerable: in a longitudinal study on 6 normozoospermia men, sperm count and TMSC increased 1.75 fold after 5 days of abstinence compared with 1 day[12]. In contrast, in most men with 2 or 3 subnormal sperm parameter values, TMSC and TNMC doubled after 1 day of abstinence compared with 2–4 days [13] . While such changes might be clinically irrelevant in normozoospermia, variations in abstinence time would represent a relatively convenient and cost-effective method to improve semen parameters and associated chances for naturally conceived or medically induced pregnancy in cases of abnormal semen parameters. Aside from the treatment of infection and possibly varicosis, there are currently very few options to increase semen quality [14]. human sperm quality. Human sperm is produced in the seminiferous tubules, and then stored in the epididymis for future release [15.16]. Unlike other species, for the male mammalian gamete to mature and acquire fertilization potential it must pass through the epididymis, where it undergoes a series of physiological and biochemical changes-15). Epididymal transit time was estimated between 2 to 11 days [16]. The variation is influenced by ejaculatory frequency (16). The World Health Organization (WHO) recommends a period of abstinence 2-7 days prior to collection for standard semen evaluation [17]. However, recent studies have suggested that shorter abstinence is better for assisted reproductive techniques (ART) than the abstinence recommended by the WHO for performing seminal diagnostic analysis. The abstinence period is important to ensure both the quantity and the quality of spermatozoa, required for successful, natural and assisted conception [18]. Long-term abstinence leads to the buildup of spermatozoa in the epididymis, and it may increase their exposure to the harmful effects of reactive oxygen and nitrogen species (ROS and RNS) generated mainly by granulocytes during maturation and storage in the epididymis. Thus, spermatozoa are susceptible to oxidative attack, and this has been correlated with decreased sperm motility, lipid peroxidation, DNA damage and compromised fertilization rates [19;20].

## Literature review

Infertility is a condition of the male or female reproductive system characterized by the inability to conceive after 12 months or more of unprotected sexual activity, [21]. Type of infertility: Infertility is classified as primary or secondary based on the existence or absence of a prior pregnancy [22,23]. Primary infertility: Is described as those who have never been able to conceive [24]. Approximately 15% of couples experience primary infertility, with male factor infertility accounting for 50% of instances. Secondary infertility: This experiment included individuals who had previously been able to conceive and bring a child [25]. Major Nomenclature of Infertile Patients

Related to Semen Quality: Normozoospermia: Normozoospermia relates to the amount, motility, and shape of sperm. Total number (or concentration, based on the stated outcome) of spermatozoa, as well as percentages of progressively motile (PR) and morphologically normal spermatozoa, at or above the lowest reference limits [26]. Causes of male infertility: Numerous underlying disorders and/or risk factors contribute to the development of male infertility. A significant increase in the risk of infertility is primarily observed in the male population [27]. Immunological causes: 5-15 percent of male infertility issues, such as cryptorchidism, primary testicular failure, testicular trauma, epididymitis, varicocele, idiopathic infertility, and infections, may have an immunological origin. Immunologic variables are regarded as a significant factor in infertility [28]. Infections: Infections of the male reproductive and urinary tracts are one of the leading causes of male infertility. Recent research indicates that UTIs account for approximately 15% of male infertility [29]. Infection and inflammation of the genital tract have been connected to 8-35 percent of male infertility cases, 30. Genetic causes: The quality of sperm may be impacted by aging, and men aged 45 and older are more likely to have DNA damage [31]. 13.7 percent of infertile men with aspermia and 4.6% of those with oligospermia have a concomitant chromosomal issue. It could be a Y chromosomal loss [32]. Approximately 1 in 650 men are affected by Klinefelter Syndrome (KS), a chromosomal disease involving the X chromosome. It is the most common hereditary cause of male infertility, and nearly all affected men are azoospermia [33]. Smoking : There are various hazardous substances in cigarette smoke, including nicotine, carbon monoxide, and cadmium, which may have negative effects on male germ cells [34]. Age: Aging can have a significant influence in male infertility; the composition and quality of seminal fluid can decline with age [35]. Age-related declines in semen characteristics, including volume, concentration, motility, and shape, have been found in men [36]. Hormonal causes : Changes in normal thyroid function led to decreased sexual activity and fertility [37]. Hypogonadism results in trophic hypogonadism, which is the most common cause of male infertility, whether acquired or congenital. Testosterone is the primary hormone responsible for spermatogenesis and sperm maturation. Testosterone fluctuations in the blood indicate hypogonadism, which affects the genital gland secretions [38]. Changes in FSH and LH hormones can be the primary cause of aberrant sperm production. Prolactin, FSH, LH, testosterone, and inhibin levels influence sperm concentration in ejaculate[39]. Alcohol : Alcohol usage has negative health and social implications. Alcoholismis associated with numerous disorders, and a relationship between alcohol consumption and male and female infertility has been proposed [39]. Nutritional factors :Recent research indicates that diet and lifestyle play a significant influence in the normal functioning of the reproductive system [40]. There is growing evidence that nutrition plays a critical role in sperm quality decline [41]. Some components of high-energy meals, including Tran's fatty acids and saturated fats, have the potential to disrupt testicular lipid metabolism and sperm production [42]. Several nutritional supplements, including carnitine, argine, zinc, selenium, and vitamin b12, have been shown to improve the number and motility of sperm [43].

## Materials and methods Population:

The study was conducted in the laboratories of the Fertility Center of Al-Sadr Teaching Hospital in Al-Najaf Governorate – Iraq between 2022 to 2023. The age of the patients ranged from 20 to 44 and the marriage period ranged from 1 to 8 years. The number of samples was ( ) . According to WHO guidelines 2021, the study samples had normozoospermia; sperm concentration was equal to or greater than 18 million, progressive motility was equal to or greater than 31%, and sperm normal

morphology was greater than 14%. Exclusion criteria includes another pathology such as oligozoospermia, asthenozoospermia and azoospermia .The correlation between the abstinence period and semen parameters was recorded.

**Semen analysis**: Infertile men were instructed to provide sperm samples via masturbation and a sexual abstinence period ranging from 2 to 7 days. The semen samples were collected in a clean container and placed in an incubator for 1 hour to complete liquefaction. Semen samples were mixed gently and well, then semen parameters were examined. Semen volume was recorded by a graduated cylinder .sperm concentration was estimated by the Makler chamber ,progressive motility was estimated by wet preparation and recorded under a light microscope (40x),sperm morphology was estimated by the eosin staining. Makler counting chamber method[44,45] A drop from a well-mixed specimen of semen was placed on the chamber and covered. The microscope with a x20 objective and x10 eyepiece was used. Counting sperm heads contained within a strip of 10 squares, a number describing sperm concentration in millions per milliliter was obtained. For the determination of sperm morphology, a smear of semen is prepared on a glass slide, air- dried, and stained with eosin/nigrosin. The slide is mounted with a coverslip and examined using a light microscope. Approximately, 200 spermatozoa per replicate are examined for normal and abnormal forms (50)(. World Health Organization . WHO laboratory manual for the examination and processing of human semen; 2010).

**Statistical Analysis:**

Data of the present study was analyzed by using SPSS Statistics Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0. IBM Corp). Comparison of differences between continuous variables was done by using the Paired t-Test when compared two means and One Way ANOVA test to compare three variables; Multiple Pairwise Comparisons were performed by using least significant differences (L.S.D). Chi squared test was performed to analyze categorized data; Pearson's correlation analysis was used to determine the relationship between variables. P values $\leq 0.05$ and $\leq 0.01$ considered as significant and highly significant.

**Results:** The study showed in table 1 sperm parameters in normozoospermia group ,the results were as the following ,abstinence period days was 4.04±1.65 , Sperm concentration million/ml was 40.22±20.36 ,Progressive motility percent was 58.08±10.04 % , Normal sperm morphology percent % was 59.80±8.18.the results of our study showed found a significant positive correlation between abstinence period with sperm concentration (r=0.24,p=0.015) while the results were revealed nonsignificant negative correlation between abstinence period with progressive motility and normal sperm morphology.

**Table 1: sperm parameters of normozoospermic samples**

| Sperm parameters | Mean±SD | Max | Min |
|---|---|---|---|
| Abstinence period ( days) | 4.04±1.65 | 2 | 15 |
| Sperm concentration million/ml | 40.22±20.36 | 15 | 140 |
| Progressive motility percent % | 58.08±10.04 | 32 | 85 |
| Normal sperm morphology percent % | 59.80±8.18 | 26 | 73 |

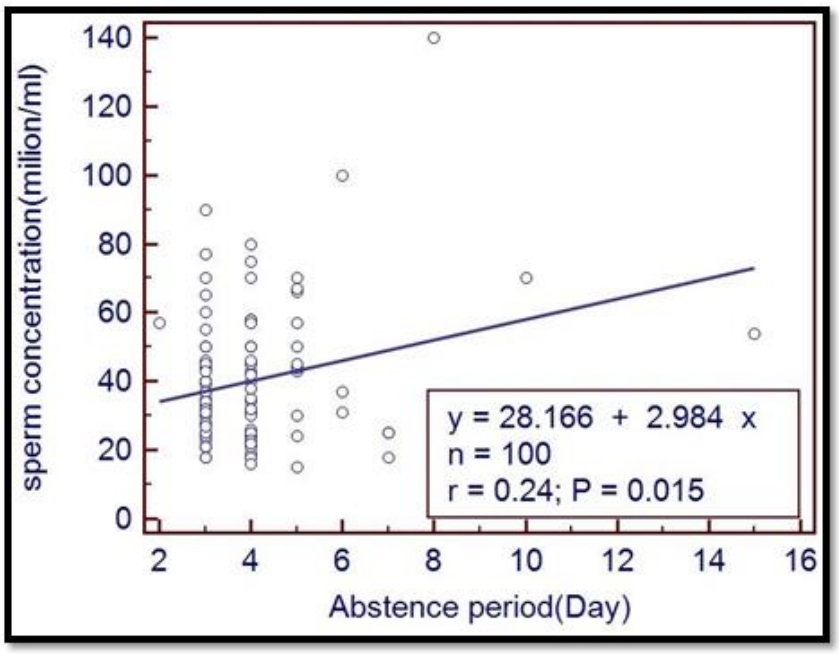


**Figure 1-correlation between abstinence period and sperm concentration in normozoospermic samples**

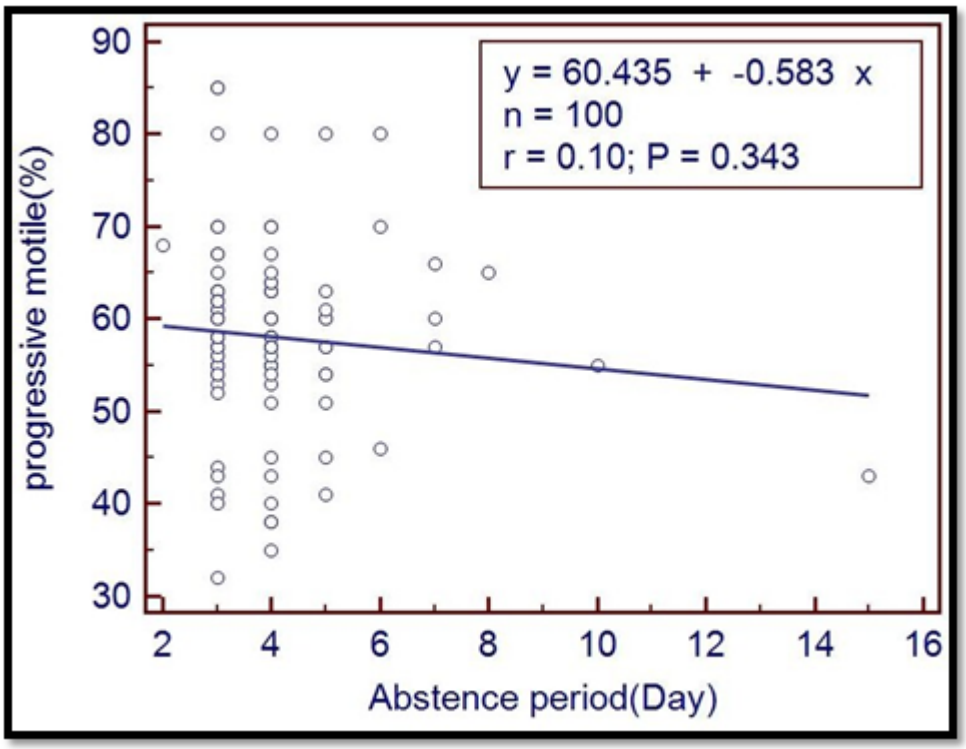


**Figure 2 - correlation between abstinence period and progressive motility percent in normozoospermic samples**

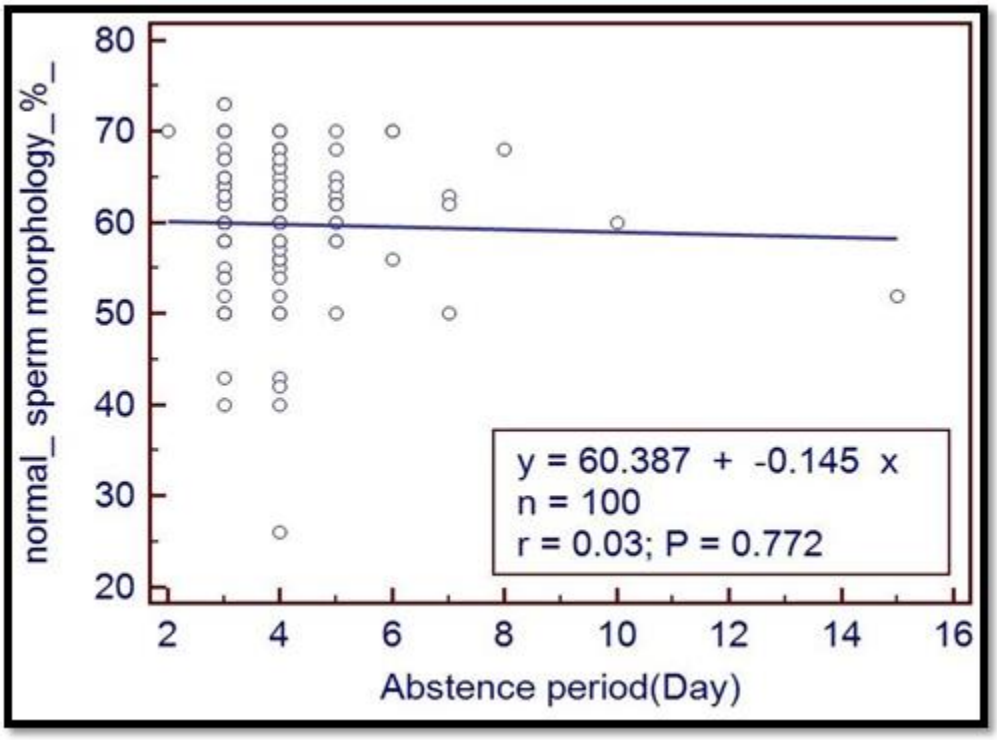


**Figure 3 - correlation between abstinence period and Normal sperm morphology percent in normozoospermia samples**

**Discussion:**

Results were as the following, abstinence period days was 4.04±1.65, Sperm concentration million/ml was 40.22±20.36, Progressive motility percent was 58.08±10.04 %, Normal sperm morphology percent % was 59.80±8.18. All results were within normal reference limited according to [46] WHO 2010 and [47] 2021. According to WHO 2021, normal sperm parameters include sperm concentration around ( 15-18 ), progressive motility around (30-32 ), and normal sperm morphology around ( 4 ) .the results showed found a positive significant correlation between abstinence and sperm concentration and may be due to accumulating of sperm in the epididymis and the continued of production sperm by spermatogenesis in the testis. Akhigbe et al., 2022 demonstrated that Prolonged (EAD) ejaculatory abstinence duration increased sperm count and total sperm count in normozoospermic patients [48].

**Conclusion:** Increase of abstinence period led to increase in sperm concentration and no impact on progressive motility and morphology in normozoospermia samples.

**Recommendation:** study the correlation between abstinence period and another variables such as leucocytes, semen volume, ROS, and, DNA fragmentation

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