

## Exploring the Impact of Paternal B12 Levels During the Periconceptional Period on IUI Success

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### KEYWORDS

Infertility, IUI, ovulation Induction, Vitamin B12, Sperm Parameters

### ABSTRACT

**Background:** The present study is a novel exploration of the effects of vitamin B12 supplementation on seminal fluid parameters and the outcomes of intrauterine insemination (IUI). Specifically, we aimed to evaluate the concentrations of vitamin B12 in blood and seminal plasma before and after two months of supplementation.

**Objectives:** investigate the effects of vitamin B12 supplementation on the characteristics of seminal fluid parameters and IUI outcomes.

**Material and Methods:** A randomized controlled experiment with 100 randomly selected infertile couples. The patients were divided into two equal groups according to their vitamin B12 dosage. Group A consists of 50 couples who are taking B12 supplements. Obtain blood and seminal plasma samples from couples to measure the level of vitamin B12. Group B consists of 50 couples and does not include any supplements. The couples in group B initiated ovulation induction and intrauterine insemination (IUI) during the same month as the medical examination without the use of any additional supplements. Still, after two months of vitamin B12 supplement, group A started ovulation induction.

**Result:** Basal semen parameters for sperm count, sperm motility in grades, and sperm morphology in the study group have a higher sperm count than the control group. Vitamin B12 intake for two months improves sperm morphology and increases sperm motility in grades A and B, according to the current study. This has a favorable effect on sperm count overall.

**Conclusion:** research will determine the positive effect of vitamin B12 supplements on sperm parameters and IUI results.

### 1. Introduction

Infertility is the term used to describe the condition when a person engages in regular, unprotected sexual activity for 12 months or more without achieving a clinical pregnancy. It is anticipated to impact 8 to 12% of couples in the reproductive age group [1]. The American Society for Reproductive Medicine has revised its definition of infertility to include the failure to achieve successful conception after engaging in unprotected sexual activity for 12 months or longer for women aged 20–34 or 6 months or longer for women aged 35 and above, without using any form of contraception [2]. According to recent research from the World Health Organization, almost 1 in 6 individuals worldwide are impacted by infertility, highlighting the prevalence of this issue. Approximately 8 to 12% of couples of reproductive ages worldwide are thought to be affected by this condition [3]. Infertility may be classified into two types: primary infertility and secondary

infertility. Primary infertility is a medical illness when a woman is unable to conceive a child, either because she cannot get pregnant or because she cannot carry a pregnancy to full term. On the other hand, secondary infertility refers to the inability to conceive after having a successful pregnancy. Infertility is a medical disorder in which a woman is unable to conceive or bring a pregnancy to term despite previously having the potential to do so [4]. Infertility may be attributed to several factors and can impact individuals of both genders. Female infertility is responsible for 20-35% of cases. Ovulatory causes are the primary cause of female infertility, often characterized by infrequent or nonexistent menstrual cycles [5]. Approximately 85% of infertility cases have a known etiology, which may be attributed to either men, females, or a combination of both. Approximately 30% of male patients with infertility did not show any apparent cause for their condition. However, 2-4% of cases were shown to be caused by defective spermatogenesis. Male variables contribute to 40-50% of all infertile study groups, and when paired with female factors, they contribute an additional 30%. Male infertility may be attributed to several conditions, such as gonadal dysgenesis, hypogonadotropic hypogonadism, varicocele, seminal tract infections, cryptorchidism, gonadal torsion or trauma, and environmental variables. Genetic factors are also crucial in this scenario. Given that obesity is a well-established factor that increases the chance of female infertility, it is recommended to include it in this list. Depending on their location, the causes of testicular problems may be classified into pre-testicular, testicular, and post-testicular categories [6], [7].

Intrauterine insemination (IUI) is an infertility treatment process. Intrauterine insemination (IUI) enhances the likelihood of conception by directly introducing carefully processed sperm into the uterus, the organ responsible for fetal development. The alternative term for the process is artificial insemination. Approximately 10-15% of couples in the globe who are of reproductive age experience infertility [8], [9]. In cases of mild male subfertility, minor endometriosis, sexual function difficulties, and unexplained infertility, the first treatment option is intrauterine insemination (IUI) [10]. When comparing IUI to in vitro fertilization (IVF), IUI is a simpler and less intrusive procedure. However, due to the increased likelihood of multiple pregnancies, there is a significant medical risk connected with it. Several factors influence the result of IUI, including maternal age, egg and sperm quality, the administration of fertility drugs, and timing. Ovarian stimulation cycles have significantly enhanced egg production and improved the success rates of IUI [11]. An IUI may be performed either in combination with ovarian stimulation or during a natural menstrual cycle. In IUI, ovarian stimulation aims to enhance the number of dominant follicles in each menstrual cycle. The underlying hypothesis is that this will result in a pregnancy rate above 20%. Adopting lifestyle adjustments is one very effective approach to improving overall health and boosting the chances of a successful IUI [10].

Vitamin B12, often known as cobalamin, is a water-soluble vitamin that plays a crucial role as a cofactor (adenosylcobalamin) in many enzymes involved in the conversion of methyl malonyl coA to succinyl coA [12]. Ultimately, food is an individual's predominant source of cobalamin. Vitamins are essential for several biological tasks, such as DNA synthesis, repair, and cell division [13]. Vitamin B12 deficiency may affect fertility in both males and females. Vitamin B12 functions as a coenzyme, facilitating the metabolism of amino and fatty acids and DNA synthesis. It benefits the number of sperm, their ability to move, the level of DNA damage in sperm, and the quality of semen. Women with a deficiency in vitamin B12 may encounter irregularities in their menstrual cycles and difficulties in the quality of ovulation [14].

Spermatogenesis is the formation of sperm cells inside the seminiferous tubules of the testes. Vitamin B12 is essential for the proper growth and upkeep of spermatogonia as they transition from mitosis to primary spermatocytes, secondary spermatocytes, spermatids, and finally mature spermatozoa (sperm cells). The positive effects of vitamin B12 on the quality of semen have been shown in around 23 published investigations, including clinical trials conducted in living organisms and experiments conducted in controlled laboratory conditions [15]. Vitamin B12 primarily improves semen quality by increasing sperm count, enhancing sperm motility, and reducing sperm

DNA damage. Therefore, semen quality is contingent upon adequate vitamin B12, often administered at the required or average doses. The positive impact of vitamin B12 on semen quality can be attributed to various factors, such as enhanced efficiency of male reproductive organs, reduced toxicity of homocysteine, increased production of nitric oxide, decreased accumulation of reactive oxygen species, reduced energy generated by spermatozoa, and diminished deterioration of semen caused by inflammation [16], [17]

This research aims to assess the levels of vitamin B12 in both blood and seminal plasma before and after a two-month period of vitamin B12 supplementation. Additionally, the study aims to examine the impact of this supplementation on seminal fluid parameters and the result of intrauterine insemination (IUI).

## **2. Methodology**

The study involves a randomized controlled experiment at the High Institute for Infertility

Diagnosis and Assisted Reproductive Technologies at AL-Nahrain University from July 2022 to March 2023. The research included a sample of 136 couples randomly chosen among the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies outpatient clinic attendees. All couples have a record of infertility after engaging in frequent unprotected sexual intercourse for one year or more. Seven individuals were pregnant at the time the supplement was used, whereas 29 individuals were unaware of the result of IUI. Only 100 instances have provided full data. The patients were categorized into two equal groups based on their use of vitamin B12 supplements. Before participation in the trial, each patient received thorough information, and all couples submitted signed informed consent. The poll was approved by the Ethics Committee, the High Institute for Infertility Diagnosis, and Assisted Reproductive Technologies.

The inclusion criteria include women and men aged between 18 and 40 years old. Mild male factor is determined as per the following: The patient exhibits a single aberrant male parameter, namely a total motile pre-wash count over 10 million per ml, a post-wash sperm count of at least 1 million, morphology  $\geq 4\%$ , and sperm motility (grade A&B)  $\geq 30\%$  of the total count cut off. Additionally, the patency of one or both fallopian tubes is confirmed. Primary or secondary infertility Typical uterine cavity Unexplained infertility While the exclusion criteria include age exceeding 40 years, both fallopian tubes are obstructed. Any untreated medical condition or pregnant contraindication, undergo infertility therapy; an ovarian cyst is a fluid-filled sac that develops in the ovary; on the day of the trigger, over three follicles were measuring more than 15mm in size, endometriosis of moderate to severe severity, acute infection of the genital tract in one or both parents, low ovarian reserve with anti-Mullerian hormone (AMH) levels of 0.5 or less and an antral follicle count (AFC) of 1 or less.

The patients were separated into two equal groups based on their supplementation of vitamin B12. Group A (B12 supplement) is shown in Fig 1. Collects couples' blood and seminal plasma samples to quantify the vitamin B12 concentration. Conduct seminal fluid analysis and perform a pelvic ultrasound to evaluate the intrafollicular count before and after administering the B12 supplement (administering a 1000 microgram oral dose of vitamin B12 vial three times per week for eight weeks for both couples). Group B (not supplement): The couple starts ovulation induction and IUI in the same month as the check-up without supplement. Other blood and seminal plasma samples are taken from couples in group A post supplement to measure vitamin B12 levels on the day of IUI, seminal fluid analysis, and U/S to assess intrafollicular count (on the second day of the cycle).

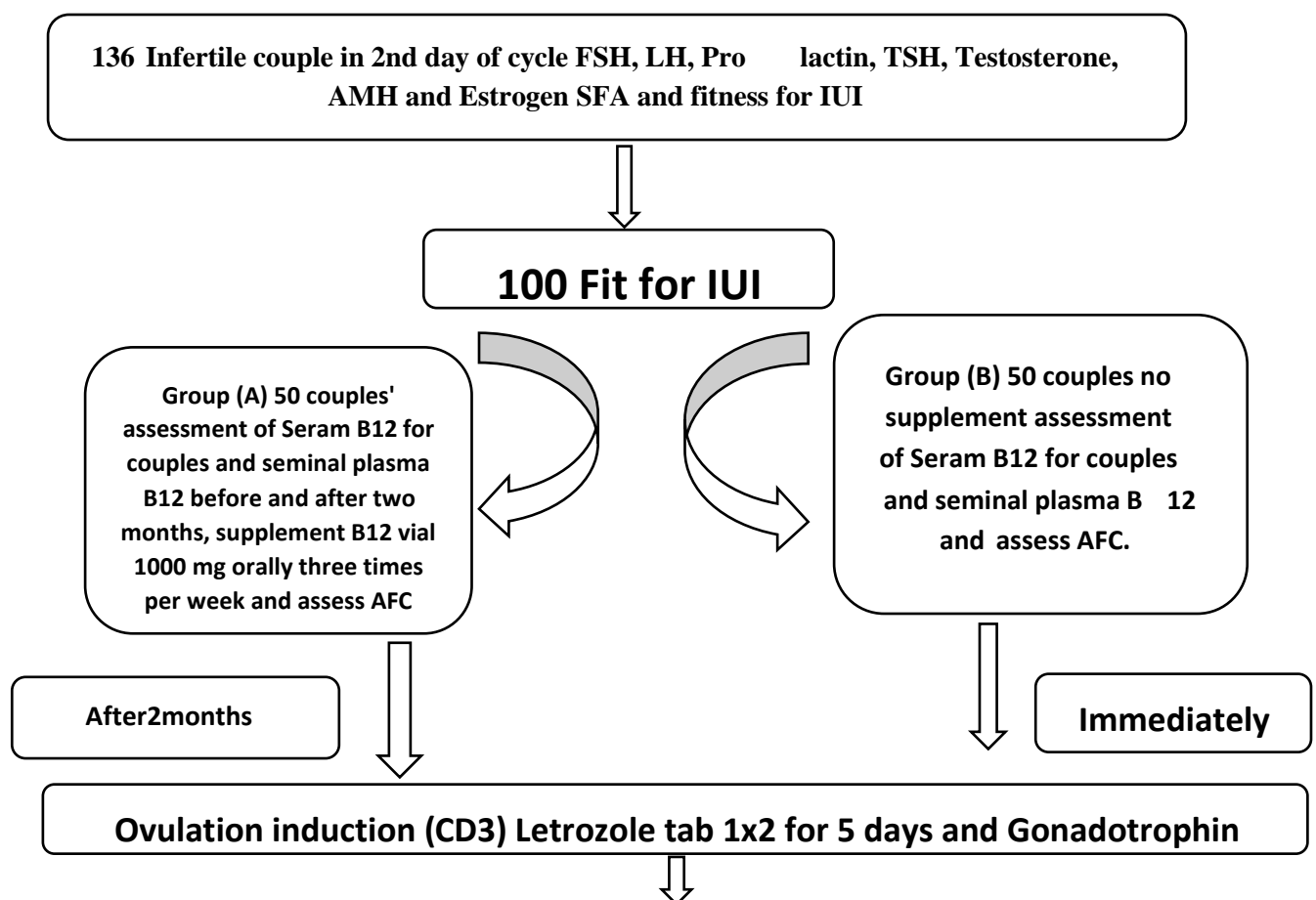
All couples who were unable to conceive were evaluated at the Assisted Reproductive Technology (ART) clinic via a comprehensive assessment of their medical history, thorough physical examination, and extensive analysis of their infertility. Each partner had a thorough evaluation of their medical history and physical condition. The comprehensive examination for the infertile pair includes the assessment of body mass index (BMI) after measuring weight and height; evaluation of hirsutism, acne, male pattern baldness, and other indications of hyperandrogenism; examination of

the thyroid gland, and inspection of the breasts for galactorrhea. Extensive investigations were conducted on both partners, including hormonal tests (such as FSH, LH, TSH, Progesterone, prolactin, E2, and AMH), viral screening tests (for hepatitis B, C, and HIV), and assessment of seminal fluid analysis (where the seminal fluid was analyzed and the results were evaluated according to the WHO 2021 standards).

This investigation used medications and kits: B12 kits for Serum and seminal plasma (Ylbiont, Shanghai-China), Bio-FSH 75IU (Biovate, York-UK), Bio-HMG 75IU (Biovate, York-UK), Cayno B12 vial 1000 (MWA-companies, Turkey), and Bio HCG 5000IU (Biovate, York-UK).

It is advisable to collect sperm samples during a period of two to five days after abstaining from any sexual activity, without doing so earlier or later. This guarantees the well-being and movement of the rapidly multiplying cells and an optimal sperm quantity. The specimen was obtained by self stimulation and then placed in a sterile, wide-mouth container where it was allowed to liquefy for about thirty minutes. Processes should be performed within ninety to one hundred twenty minutes after sample collection, commencing after the substance has become liquid. Before the intrauterine insemination (IUI) technique, a semen sample is collected and purified. During the process of sperm wash, any surplus fluid is extracted, along with any weak or deceased sperm cells, pathogens, debris, and prostaglandins that might potentially cause uterine cramping. Furthermore, this procedure concentrates the most mobile sperm cells in a single location, hence augmenting the number of motile sperm cells that reach the uterus. This often happens on the day that intrauterine insemination (IUI) is carried out.

To acquire a concentrated sample of healthy sperm, the sperm undergo a process known as "washing" and filtration.



When the dominant follicle reaches 17 mm, give trigger (HCG 5000IU)

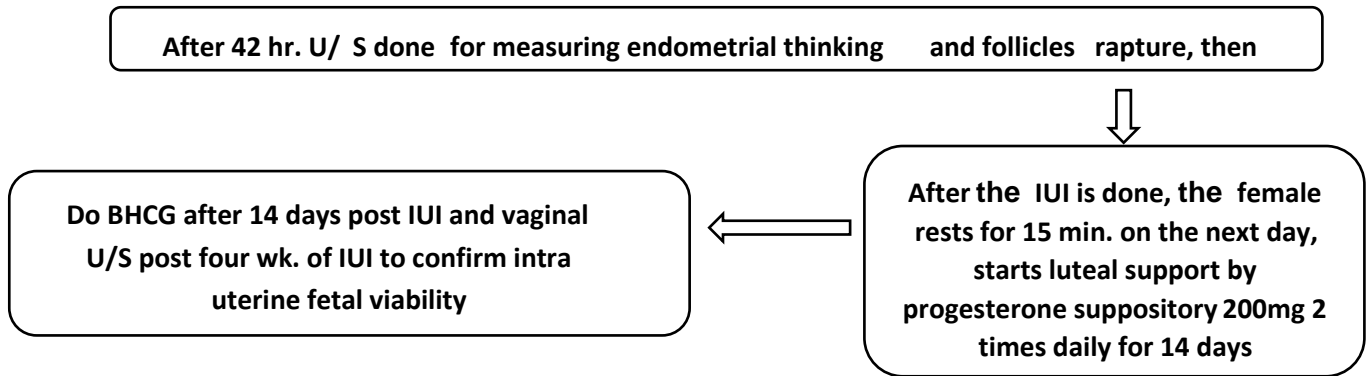


Figure 1 study design

### Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft Office 2010. The data was described using descriptive statistics, including frequency, mean, and standard errors. The groups were compared using an independent sample t-test to compare the two groups, a chi-square to compare non-continuous variables or percentages, and a paired sample t-test to compare continuous variables in two periods, namely before and after. The findings were deemed statistically significant if the value was 0.05 or below

### 3. Results and Discussion

#### Results Age and BMI comparisons between the study and control groups

The results were presented as the mean plus or minus the standard error of the mean (SEM). In terms of the mean male's age ( $33.38 \pm 0.97$  vs.  $32.10 \pm 0.97$ ;  $p=0.353$ ), mean female's age ( $27.86 \pm 0.67$  vs.  $28.84 \pm 0.87$ ;  $p=0.375$ ), mean male's body mass index (BMI) ( $28.93 \pm 0.39$  vs.  $29.96 \pm 0.41$ ;  $p=0.071$ ) and mean female body mass indices ( $27.33 \pm 0.52$  vs.  $26.98 \pm 0.51$ ;  $p=0.630$ ), as well as mean male's age ( $27.86 \pm 0.67$  vs.  $28.84 \pm 0.87$ ), all these illustrated in figure 2.

#### Study and control groups compared for the length, nature, and sources of infertility

Regarding the duration of infertility, there were no significant differences between the study and control groups ( $4.81 \pm 0.45$  vs.  $5.02 \pm 0.48$ ;  $p=0.751$ ). Similarly, there were no significant differences in the primary or secondary type of infertility ( $p=0.072$ ), and factors such as male and female causes, unexplained causes, combined causes, and other causes of infertility ( $p=0.053$ ) were also not different, presented in figure 3.

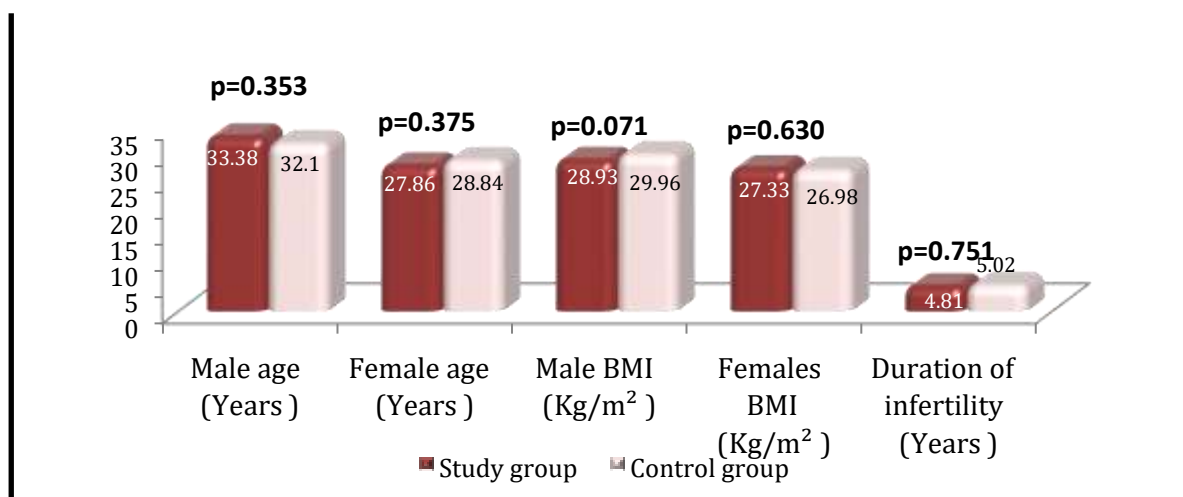
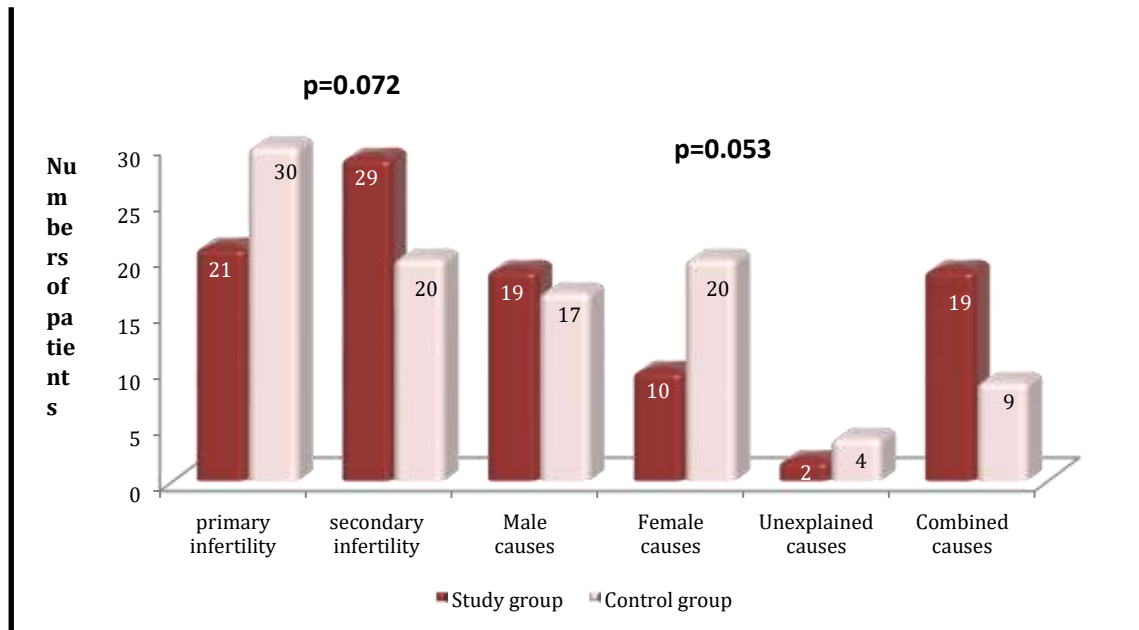


Figure 2 Comparison of patient's age, BMI & duration of infertility between study & control group.



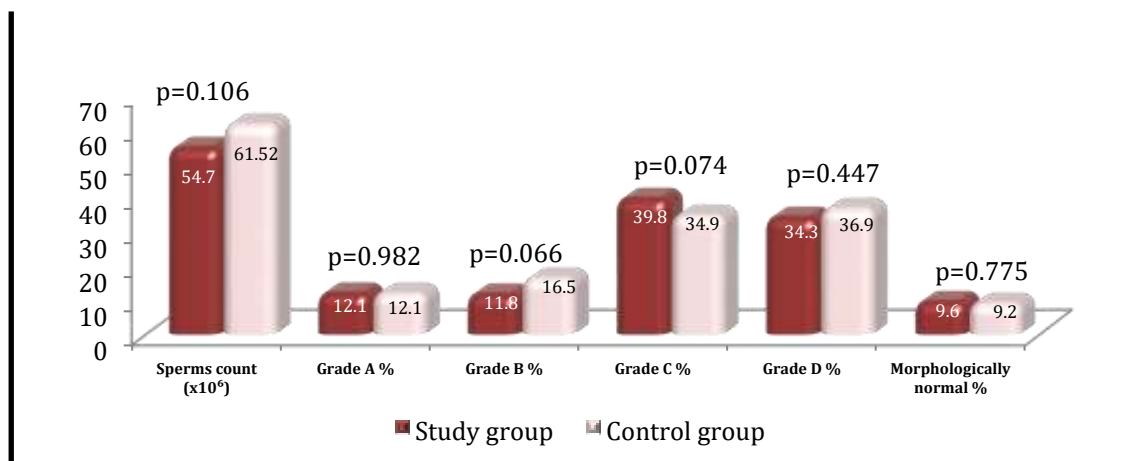


**Figure 3 Comparison of the type and causes of infertility between study & control groups**

#### Comparison of basal seminal fluid parameters in the study and control groups

Basal semen parameters for sperm count were similar across study and control groups

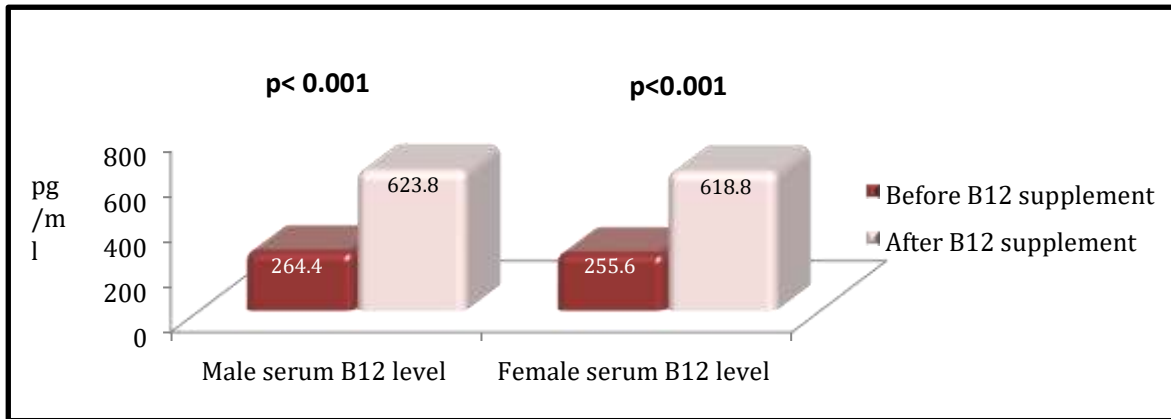
( $p=0.106$ ), grade A sperm motility ( $p=0.982$ ), grade B ( $p=0.066$ ), grade C ( $p=0.074$ ), and grade D ( $p=0.447$ ) in terms of shape standard percentage of sperm ( $p=0.775$ ), as shown in Figure4.



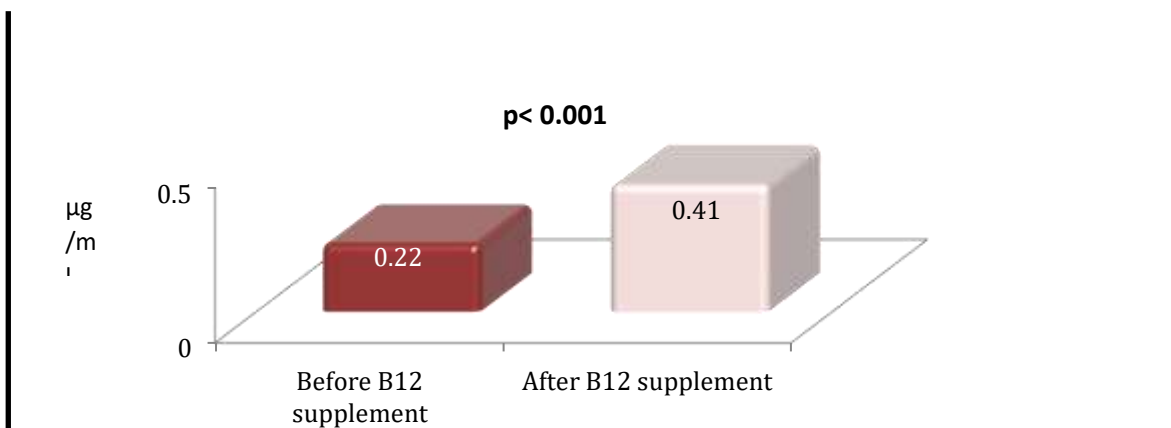
**Figure Error! No text of specified style in document. Comparison of basal seminal fluids parameters between study and control groups**

#### Comparison of B12 levels between research and control groups before and after vitamin B12 supplementation

There were no significant changes seen between the study and control groups in terms of serum and seminal plasma B12 levels in males before the administration of vitamin B12 supplements ( $264.4 \pm 16.41$  vs.  $289.5 \pm 8.07$ ;  $p=0.173$ ), ( $0.22 \pm 0.02$  vs.  $0.24 \pm 0.01$ ;  $p=0.322$ ) correspondingly (figure 5 and 6).



**Figure 5 Comparison of B12 levels between study and control groups**



**Figure 6 Comparison of seminal B12 levels before and after B12 supplement.**

### Comparison of seminal fluid analytical parameters before and after B12 supplementation (using prewashed samples) in the research group

The semen sperm count was markedly elevated ( $54.70 \pm 2.64$  vs.  $154.26 \pm 6.67$ ;  $p < 0.001$ ), percentage of Grade A sperm ( $12.10 \pm 0.96$  vs.  $21.60 \pm 1.37$ ;  $p < 0.001$ ), percentage of grade B sperm ( $11.80 \pm 1.18$  vs.  $17.00 \pm 1.12$ ;  $p < 0.001$ ) and morphologically standard sperms percent ( $9.61 \pm 0.23$  vs.  $10.81 \pm 0.15$ ;  $p < 0.001$ ) among the participants who got a vitamin B12 supplement, there was a substantial decrease in the percentage of grade C sperms ( $39.88 \pm 1.56$  vs.  $32.70 \pm 1.77$ ;  $p = 0.003$ ) and the percentage of grade D sperm ( $34.32 \pm 2.29$  vs.  $28.20 \pm 1.72$ ;  $p = 0.002$ ) (Table 1).

**Table 1: Comparison of seminal fluids analysis parameter pre-sperm washing among the study group before and after B12 supplementation**

| Parameters (Mean $\pm$ SE) |          | Before B12 supplement | After B12 supplement | p-value     |
|----------------------------|----------|-----------------------|----------------------|-------------|
| Sperms count ( $10^6$ )    |          | $54.70 \pm 2.64$      | $154.26 \pm 6.67$    | < 0.001 P S |
| Sperms motility            | Grade A% | $12.10 \pm 0.96$      | $21.60 \pm 1.37$     | < 0.001 P S |
|                            | Grade B% | $11.80 \pm 1.18$      | $17.00 \pm 1.12$     | < 0.001 P S |
|                            | Grade C% | $39.88 \pm 1.56$      | $32.70 \pm 1.77$     | 0.003 P S   |
|                            | Grade D% | $34.32 \pm 2.29$      | $28.20 \pm 1.72$     | 0.002 P S   |
| Normal sperms morphology % |          | $9.61 \pm 0.23$       | $10.81 \pm 0.15$     | < 0.001 P S |

P: Paired sample t-test; S: Significant ( $p \leq 0.05$ )

### Comparison of seminal fluids parameters pre-sperm processing between study group after two months of B12 supplement and control group

Comparison of seminal fluids parameters pre-sperm processing between study and control groups was demonstrated in Table 2 according to the results, the study group showed significantly higher sperms count ( $154.26 \pm 6.67$  vs.  $94.52 \pm 6.83$ ;  $p < 0.001$ ) and grade A sperms percent ( $21.60 \pm 1.37$  vs.  $12.14 \pm 1.48$ ;  $p < 0.001$ ), on the other hand, there was significantly lower grade D sperms percent among the study group ( $28.20 \pm 1.72$  vs.  $36.96 \pm 2.59$ ;  $p = 0.006$ ). There were also no significant differences concerning grade B sperms ( $p = 0.862$ ), grade C sperms percent ( $p = 0.447$ ), and morphologically normal sperms ( $p = 0.257$ ).

**Table 2 Comparison of seminal fluids parameters pre-sperm processing samples between study and control group**

| Pre sperms processing parameters |          | Study group (Mean $\pm$ SE)         | Control group (Mean $\pm$ SE)      | <i>p-value</i>        |
|----------------------------------|----------|-------------------------------------|------------------------------------|-----------------------|
| Sperms count (106/ml)            |          | <b>154.26 <math>\pm</math> 6.67</b> | <b>94.52 <math>\pm</math> 6.83</b> | <b>&lt; 0.001 F S</b> |
| Sperms motility                  | Grade A% | 21.60 $\pm$ 1.37                    | 12.14 $\pm$ 1.48                   | < 0.001 F S           |
|                                  | Grade B% | 17.00 $\pm$ 1.12                    | 16.56 $\pm$ 2.27                   | 0.862 F NS            |
|                                  | Grade C% | 32.70 $\pm$ 1.77                    | 34.90 $\pm$ 2.27                   | 0.447 F NS            |
|                                  | Grade D% | 28.20 $\pm$ 1.72                    | 36.96 $\pm$ 2.59                   | 0.006 F S             |
| Normal sperms                    |          | 10.81 $\pm$ 0.15                    | 9.20 $\pm$ 1.41                    | 0.257 F NS            |

F: Independent sample t<sup>morphology</sup> -test; NS: Not significant ( $p > 0.05$ ) S: Significant ( $p \leq 0.05$ )

**Comparison of seminal fluid parameters following sperm washing between the study and control groups** the study group showed a significantly higher sperm count ( $72.40 \pm 2.27$  vs.  $57.78 \pm 3.65$ ;  $p = 0.001$ ) and a higher percentage of morphologically normal sperm ( $79.50 \pm 1.14$  vs.  $61.50 \pm 2.64$ ;  $p < 0.001$ ) after sperm washing. However, there was no significant difference in sperm motility grades between the study and control groups ( $p > 0.05$ ) (Table 3).

**Table 3: Comparison of seminal fluids parameters after sperms washing between study and control groups**

| Parameters                      |          | Study group (Mean $\pm$ SE) | Control group (Mean $\pm$ SE) | <i>p-value</i> |
|---------------------------------|----------|-----------------------------|-------------------------------|----------------|
| Sperms count (10 <sup>6</sup> ) |          | 72.40 $\pm$ 2.27            | 57.78 $\pm$ 3.65              | 0.001*         |
| Sperms                          | Grade A% | 32.86 $\pm$ 1.70            | 33.50 $\pm$ 3.17              | 0.859          |
|                                 | Grade B% | 24.60 $\pm$ 1.20            | 21.70 $\pm$ 2.02              | 0.221          |
|                                 | Grade C% | 20.90 $\pm$ 1.37            | 20.16 $\pm$ 150               | 0.716          |
|                                 | Grade D% | 22.24 $\pm$ 1.83            | 24.34 $\pm$ 2.37              | 0.484          |
| Normal sperms morphology        |          | 11.39 $\pm$ 0.17            | 10.11 $\pm$ 0.16              | < 0.001**      |

F: Independent sample t-test; \* significant difference \*\* Highly Significant ( $p < 0.001$ )

### Analysis of pre- and post-supplement B12 levels in the study group

The levels of vitamin B12 were considerably elevated following the administration of a vitamin B12 supplement in relation to the levels found in male serum ( $264.4 \pm 16.41$  vs.  $623.8 \pm 28.68$ ;  $p < 0.001$ ) and male seminal plasma ( $0.22 \pm 0.02$  vs.  $0.41 \pm 0.01$ ;  $p < 0.001$ ) (Table 4).



**Table 4: Comparison of B12 levels before and after B12 supplements among the study group**

| Parameters<br>(Mean±SE)  | Before B12<br>supplement | After B12<br>supplement | <i>p-value</i> |
|--------------------------|--------------------------|-------------------------|----------------|
| Males' serum B12 (pg/ml) | 264.4 ± 16.41            | 623.8 ± 28.68           | < 0.001**      |
| Seminal plasma<br>B12    | 0.22 ± 0.02              | 0.41 ± 0.01             | < 0.001**      |

P: Independent sample t -test; \*\*Highly Significant (p <0.001)

#### Relationship between research group members' blood and seminal plasma levels of vitamin B12 and semen parameters

Among patients in the study group, there were no statistically significant associations found between serum and seminal vitamin B12 levels and semen analytical parameters (Table 5)

**Table 5: Correlation between serum and seminal B12 with semen parameters among the study group**

| Parameters                      | Statistics     | Serum B12 level | Seminal B12 levels |
|---------------------------------|----------------|-----------------|--------------------|
| Sperms count (10 <sup>6</sup> ) | R              | 0.122           | 0.011              |
|                                 | <i>p-value</i> | 0.397 NS        | 0.937              |
| Grade A%                        | R              | - 0.088         | 0.005              |
|                                 | <i>p-value</i> | 0.957 NS        | 0.970              |
| Grade B%                        | R              | - 0.006         | - 0.003            |
|                                 | <i>p-value</i> | 0.965 NS        | 0.985              |
| Grade C%                        | R              | - 0.068         | - 0.094            |
|                                 | <i>p-value</i> | 0.638 NS        | 0.517              |
| Grade D%                        | R              | 0.123           | 0.051              |
|                                 | <i>p-value</i> | 0.396 NS        | 0.726              |
| Normal sperms morphology %      | R              | - 0.182         | - 0.143            |
|                                 | <i>p-value</i> | 0.206 NS        | 0.321              |

R: person correlation

#### Comparison of study group B12 levels based on pregnancy outcome

Before and after vitamin B12 supplementation, there are no significant changes (p > 0.05) in male and female seminal and serum B12 levels between couples with positive and negative pregnancies (Table 6)

**Table 6: Comparison of B12 levels according to pregnancy outcome among the study group**

| Parameters<br>(Mean±SE)                   | Positive<br>pregnancy | Negative<br>pregnancy | p-value |
|---|-----------------------|-----------------------|---------|
| Males' serum B12 before<br>B12 supplement | 291.3 ± 40.10         | 253.9 ± 16.74         | 0.311   |

|  |                   |                   |       |
|--|-------------------|-------------------|-------|
| <b>Males' serum B12 after B12 supplement</b> | 677.0 $\pm$ 53.69 | 603.1 $\pm$ 33.75 | 0.252 |
| <b>Semen B12 before B12 supplement</b>       | 0.19 $\pm$ 0.01   | 0.23 $\pm$ 0.03   | 0.508 |
| <b>Semen B12 after B12 supplement</b>        | 0.41 $\pm$ 0.01   | 0.41 $\pm$ 0.01   | 0.911 |

Independent sample t-test

## Discussion

This study examines vitamin B12 with infertility in men using numerous factors and IUI results. Vitamin B12's link to serum and seminal plasma vitamin levels and alterations in male seminal fluid parameters is little studied. The present study found that there were no statistically significant differences in the average age of couples, body mass index (BMI) in males and females, primary or secondary type of infertility, and causes of infertility (including male causes, female causes, unexplained causes, and combined causes) between the group receiving vitamin B12 supplementation (Study group) and the control group. This lack of difference can be attributed to the fact that both groups were selected based on the same inclusion and exclusion criteria. The researchers ensured that the parameters of ovulation, sperm parameters, and pregnancy outcomes were equally distributed among the participants to prevent bias in the study's results. Our thorough data showed no notable variations in the basal semen parameters between the study and control groups pre-treatment. This encompasses essential factors such as the amount of semen, the concentration of sperm, the quality of sperm movement, and the shape of sperm. These findings provide further evidence to reinforce the dependability of the outcomes obtained from our investigation. The extensive comparisons of B12 levels before vitamin B12 supplementation in the serum of males, seminal plasma, and females' serum demonstrate a consistent and comforting pattern. The study and control groups did not exhibit any notable disparities, further reinforcing our results' dependability.

The study's findings revealed a notable increase in sperm concentration, sperm motility (percentage of grade A and grade B sperm), and morphologically normal sperm among participants who were given vitamin B12 supplements. Conversely, this group of patients experienced a significant decrease in the percentage of grade C and grade D sperm. This finding is consistent with another investigation [16], [18]. Studies have shown that administering vitamin B12 boosts the quantity of motile sperm in both human and animal subjects. Improved sperm movement: Research has shown that vitamin B12 enhances sperm movement in males with infertility issues and promotes the development of normal sperm structure. The observed outcomes might be attributed to reducing oxidative stress and improving energy production. Additionally, B12 safeguards sperm health by controlling homocysteine levels. Vitamin B12 may mitigate inflammation in the male reproductive system, potentially improving sperm quality. A separate study contradicts our findings by indicating no substantial association between sperm morphology and vitamin B12 content. However, it does support our conclusion of an elevation in sperm concentration [19], [20].

During the study, it was found that post-processing sperm samples exhibited a significantly higher percentage of sperm motility in grade A, while there was no significant difference in normal sperm morphology and sperm motility in grade B. Additionally, there was a considerably lower total sperm count and percentage of sperm motility in grades C and D. This finding is consistent with previous research conducted in the same field [21]. All evaluated sperm parameters were significantly modified by processing. Improvements were seen in sperm concentration, percentage of motile sperm, concentration of motile sperm, and other related metrics. Additional research corroborates our

findings [22]. indicating an augmentation in sperm concentration per ml and an improvement in normal morphology. After processing, the study group had higher sperm counts and more morphologically normal sperm. There was no significant difference in sperm motility grades between the study and control groups. Male serum, male seminal plasma, and female serum B12 levels increased significantly after vitamin B12 supplementation. The findings indicate a rise in vitamin B12 levels in both blood and seminal plasma and enhancements in sperm quality and parameters. However, no noteworthy associations were seen among the patients in the research group. Contrary to this finding, several studies challenge it by gathering data from research published between 2011 and 2021 on human subjects. Research also demonstrates that vitamin B12 enhances semen quality by increasing both the movement and quantity of sperm while simultaneously reducing the level of DNA damage in sperm. This highlights the advantages of vitamin B12 in enhancing semen quality by increasing sperm count, improving sperm motility, and reducing sperm DNA damage. The disparity in this outcome may be attributed to the limited size of the sample [23], [24]

#### 4. Conclusion and future scope

The present research and findings indicate that Vitamin B12 supplementation for two months positively impacts sperm count, particularly in increasing sperm motility in grades A and B and improving sperm morphology. The efficacy of a vitamin B12 supplement in the IUI program requires further investigation via prospective randomized control studies to determine the optimal dosage of vitamin B12. Additionally, many IUI trials should be conducted to validate the findings. To accurately assess the impact of vitamin B12 on conception rates, a more extensive sample size and a longer study duration are required, including both natural and artificial reproductive methods.

#### Reference

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