IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Mouroge et al., Vol. 14, Issue 2, Pp. 70-84 (2024) Original Research
DOI: http://doi.org/10.28969/IJEIR.v14.i2.r8.24 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Vitamin B12 Levels in Infertile Couples and Their Effect on the Performance of IUI

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ABSTRACT

The research objective was to evaluate the blood vitamin B12 levels in females before and after two months of vitamin B12 supplementation. Additionally, the study intended to investigate the effects of this supplementation on antral follicle count (AFC), the number of dominant follicles (DF), the outcome of intrauterine insemination (IUI), and the effect of the outcome by age, and BMI, cause of infertility and types of infertility .

A randomized controlled trial was undertaken, including 100 couples that have provided comprehensive data. Segregated into two equivalent groups based on their vitamin B12 intake. Groups A and B, group. Group A comprises 50 pairs of individuals using B12 supplements for eight weeks, and Group B contains no additional elements. Collect blood and seminal plasma samples from all couples to assess their vitamin B12 levels. The two groups underwent ovulation induction and intrauterine insemination (IUI) concurrently with the medical evaluation. Group A started after two months of supplement, but Group B began immediately.

Vitamin B12 increases female blood levels, antral follicle count, and dominant follicles (DF). The pregnancy rate has little effect. The antral follicle count and numbers of dominant follicles did not differ between pregnant and non-pregnant women after vitamin B12 supplementation.

How to cite:

Mouroge Khalaf Atea , Laith Amer Al-Anbari , Vitamin B12 Levels in Infertile couple and Their Effect on the Performance of IUI; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2024); 14(2).
Doi: http://doi.org/10.28969/IJEIR.v14.i2.r8.24

KEYWORD

AFC, DF, Infertility, IUI, BMI.

1. Introduction

Infertility refers to the inability to achieve a clinical pregnancy while engaging in frequent, unprotected sexual activity for 12 months or more(1). This condition affects nearly 1 in every seven couples(2). The American Society for Reproductive Medicine has updated its definition of infertility to encompass the inability to achieve successful conception after engaging in unprotected sexual activity for 12 months or more for women aged 20–34 or 6 months or more for women aged 35 and above, without employing any form of contraception(3,4). A recent study by the World Health Organization reveals that about one-sixth of the global population is affected by infertility, underscoring the widespread nature of this problem. This illness is estimated to afflict about 8 to 12% of couples in their reproductive years globally(5). Infertility may be categorized into two distinct types: primary infertility and secondary infertility. Primary infertility is a medical condition in which a woman is unable to achieve pregnancy, either due to an inability to conceive or an inability to sustain a pregnancy to its completion. Secondary infertility is a medical condition characterized by the failure of a woman to conceive or carry a baby to full term, although having previously had the capability to do it(6) (7). The causes of infertility may be identified in around 85% of instances; these causes might be male- or female-specific or even a mix of the two. Thirty percent of infertile men had no apparent medical reason for their problem. On the other hand, abnormal spermatogenesis was shown to cause 2-4% of instances. For infertile research groups, male variables account for 40-50% of the total. When combined with female factors, they account for an extra

30%. Environmental factors, cryptorchidism, gonadal torsion or trauma, varicocele, hypogonadotropic hypogonadism, seminal tract infections, and gonadal dysgenesis are among the many possible causes of male infertility. 20-35% are caused by female infertility. Infertility in women is most often caused by problems with ovulation, which manifests itself in irregular or nonexistent menstrual periods(8,9).

Genetic factors play a significant role in this situation. Obesity is a well-recognized risk that heightens the likelihood of female infertility(10).

Intrauterine insemination (IUI) is a medical procedure used to treat infertility. Intrauterine insemination (IUI) increases the probability of conception by directly delivering meticulously treated sperm into the uterus, the organ responsible for the development of the fetus. Approximately 10-15% of couples worldwide who are in the age range of being able to have children have infertility(11,12). IUI is the first therapy choice for moderate male subfertility, mild endometriosis, sexual function issues, and unexplained infertility(13). When comparing intrauterine insemination (IUI) to in vitro fertilization (IVF), IUI is a less complex and less invasive treatment. Nevertheless, the higher probability of having multiple pregnancies is associated with a substantial medical hazard. Various variables impact the outcome of IUI, such as the age of the mother, the quality of the egg and sperm, the use of fertility medications, and the scheduling of the procedure. Ovarian stimulation cycles have substantially increased the number of eggs and raised the success rates of IUI(14). IUI may be conducted either with ovarian stimulation or during a regular menstrual cycle. Ovarian stimulation in IUI aims to increase the number of dominant follicles in each menstrual cycle. The fundamental assumption is that this will lead to a pregnancy rate above 20%. One very productive strategy for enhancing general health and increasing the likelihood of successful intrauterine insemination (IUI) is implementing lifestyle modifications.

Vitamin B12, sometimes referred to as cobalamin, is a kind of vitamin that dissolves in water and serves as an essential cofactor (adenosylcobalamin) in several enzymes that participate in the conversion of methyl malonyl coA to succinyl CoA (15). Ultimately, food is the primary source of cobalamin for humans. Vitamins are necessary for several biological processes, including DNA synthesis, repair, and cell division(16). Insufficient levels of Vitamin B12 may impact the reproductive abilities of both men and women. Vitamin B12 acts as a coenzyme, aiding in the breakdown of amino and fatty acids and the production of DNA. It positively impacts sperm count, motility, DNA integrity, and semen quality(17). Women who have a shortage of vitamin B12 may have abnormalities in their menstrual periods and challenges in the quality of ovulation(18).

Oogenesis is the process of ovum formation, which is distinct from spermatogenesis in some aspects. While the gamete produced by spermatogenesis is primarily a mobile nucleus, the gamete created through oogenesis has all the necessary substances to commence and sustain metabolism and growth. Hence, oogenesis forms a haploid nucleus and accumulates cytoplasmic enzymes, mRNAs, organelles, and metabolic substrates. As the sperm undergoes differentiation to acquire motility, the egg develops a very intricate cytoplasm(19). Vitamin B12 is crucial for the growth and multiplication of oocytes during oogenesis. When caffeine was ingested, a study found that it hindered the growth of egg cells by disrupting the structural development of mitochondria in the egg cells(20). Vitamin B12 has a beneficial effect on the process of egg yolk formation and the reaction of mitochondria to stress during the development of eggs. Moreover, the addition of vitamin B12 counteracted the harmful effects of caffeine on reproduction by increasing the amounts of oocyte yolk protein and reducing mitochondrial oxidative stress. According to this study, the injection of vitamin B12 protects against the process of vitellogenesis and oocyte development by maintaining the activity of mitochondria in Caffeine Ingestion by Animals CIA (20). Women who are unable to conceive have significantly lower amounts of vitamin B12. Vitamin B12 deficiency has been shown to cause infertility by disrupting cell division in the fertilized egg, preventing ovulation, and impairing implantation owing to changes in endometrial structure caused by abnormal DNA synthesis. Vitamin B12 is essential for the production of DNA and the division of cells. It has a crucial role in promoting rapid cell proliferation and the maturation of oocytes(21).

This research aimed to assess the blood vitamin B12 level in an infertile couple before and after two months of vitamin B12 supplementation and its effect on IUI (intrauterine insemination) results.

2. Patients and Methods

The research conducted a randomized controlled trial at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at AL-Nahrain University from July 2023 to March 2024. The study included a cohort of 100 couples selected randomly from the High Institute for Infertility Diagnosis and Assisted

Reproductive Technologies outpatient clinic attendees. All couples with a history of infertility after participating in regular unprotected sexual intercourse for one year or longer. Before enrolling in the experiment at the Assisted Reproductive Technology (ART) clinic, couples who were unable to conceive had a complete evaluation that included a detailed review of their medical history, a thorough physical examination, and an exhaustive study of their infertility. Every partner underwent a comprehensive assessment of their medical background and physical state. The comprehensive examination for the infertile couple involves measuring the body mass index (BMI) by assessing weight and height, evaluating hirsutism, acne, male pattern baldness, and other signs of excessive male hormones, examining the thyroid gland, and checking the breasts for galactorrhea. Thorough investigations were carried out on both individuals, which involved conducting hormonal tests at day two of a cycle (such as FSH, LH, TSH, Progesterone, prolactin, E2, and AMH), viral screening tests (for hepatitis B, C, and HIV), and evaluating the quality of the seminal fluid parameter based on the WHO 2021 standards) shown in Table (1)(22) 20. every patient was provided with comprehensive information, and all couples provided written informed consent. The survey received approval from the Ethics Committee, the High Institute for Infertility Diagnosis, and Assisted Reproductive Technologies.

The inclusion criteria include females within the age range of 18 to 40 years. The determination of mild male factor is based on the following criteria: The patient shows one abnormal male parameter, namely a pre-wash count of over 10 million motile sperm per milliliter, a post-wash sperm counts of at least 1 million, morphology ≥4%, and sperm motility (grade A&B) ≥ 30% of the total count threshold. Furthermore, the patency of either one or both fallopian tubes is confirmed.

Either primary or secondary infertility. The standard anatomical structure of the uterus. Unexplained infertility is a condition when the cause of infertility cannot be determined. The exclusion criteria for this condition include being above the age of 40 and having both fallopian tubes blocked. An untreated medical condition or pregnancy contraindication can hinder infertility therapy. An ovarian cyst is a fluid-filled sac that forms in the ovary. On the trigger day, more than three follicles measured over 15mm. Endometriosis of moderate to severe severity, acute infection of the genital tract in one or both parents and low ovarian reserve with anti-Mullerian hormone (AMH) levels of 0.5 or less, and an antral follicle count (AFC) of 1 or less are also factors to consider.

The patients were divided into two equal groups depending on their vitamin B12 supplement, as shown in the study design (Figure 1). Group A (supplemented with vitamin B12): Obtain serum, seminal plasma samples from couples, and seminal fluid analysis before starting the supplement and after two months of vitamin B12 intake to measure the level of vitamin B12, its effect on seminal fluid parameters, AFC, DF, and pregnancy rate post-IUI After providing both spouses 1000 micrograms of an oral dose of vitamin B12 vial thrice weekly for eight weeks, Group B begins ovulation induction and intrauterine insemination (IUI) in the same month as the check-up without using supplementary medications. On intrauterine insemination (IUI) day, blood and seminal plasma samples are collected from couples to examine vitamin B12 levels using a human vitamin B12 ELISA kit to measure VB12 in human serum and seminal plasma; prepare all reagents, samples, and standards and add samples with reagents to ELASA solution and lats to react together for 60 min. at 37c. Wash the plate, add chromogen solutions A and B, and incubate for ten min. at 37c to develop the color; add stop solution, read the absorbance concentration within ten min., and calculate) 21. Seminal fluid analysis and ultrasound (U/S) are also performed on the second day of the menstrual cycle to evaluate the intrafollicular count.

The inquiry utilizes many medications and kits, including B12kits for Seram 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). On the second day of the menstrual cycle, women are positioned in the dorsal lithotomy position with an empty bladder. A vaginal ultrasound is performed during the early follicular phase to count the antral follicles (figure 3), measure the thickness of the endometrium, and check for the presence of ovarian cysts or any other conditions that may hinder intrauterine insemination. On the seventh day, a second ultrasound was conducted to assess the size and number of dominant follicles and measure the endometrium thickness of the endometrium (Figure 4). The endometrial thickness was calculated using a triple line pattern, and an endometrial thickness (ET) measurement was taken in all couples, falling within the 7-12mm range. Before commencing the IUI program, it is necessary to do a Hysterosalpingogram (HSG), laparoscopy, or hysterosalpingo contrast sonography (HYCOSY) to assess the patency of the fallopian tubes.

Using ultrasound, ovulation induction is performed after assessing endometrial thickness and antral follicle count (AFC). The induction is done by administering the aromatase inhibitor tablet (letrozole 2.5 mg) orally twice daily from the third day of the cycle for five days and Gonadotrophin injection accordingly. After the ultrasound assessment for the number and size of the dominant follicle and endometrial thickening, the trigger was executed and performed on the designated day, and an HCG injection of 5000 IU was administered. The intrauterine insemination (IUI) procedure was performed 42 hours after administering the ovulation trigger and preparing the sperm after a vaginal ultrasound luteal phase support by taking progesterone suppository for 14 days .

Beta human chorionic gonadotropin estimation 14 days after IUI is done; in positive cases, the progesterone suppository continues until the end of the first trimester, and in negative cases, it should be stopped immediately; U/S should be done two weeks later to confirm pregnancy by the presence the gestational sac and fetal pole with fetal heart

Committee

Table (1) seminal fluid analysis according to WHO2021

Semen volume (ml)	1.4(1.3-1.5)
Total sperm number (10^6per ejaculation)	39(35-40)
Total motility (%)	42(40-43)
Progressive motility (%)	30(29-31)
Non-progressive motility (%)	1
Immotile sperm (%)	20(19-21)
Normal form (%)	4(3.9-4)
Vitality (%)	54(50-56)

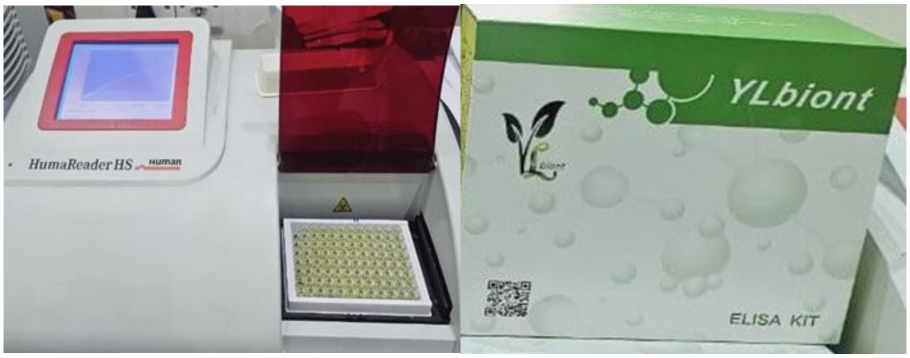

Figure (1) showB12kit equipment

Figure (2): The intrafollicular count in the follicular phase

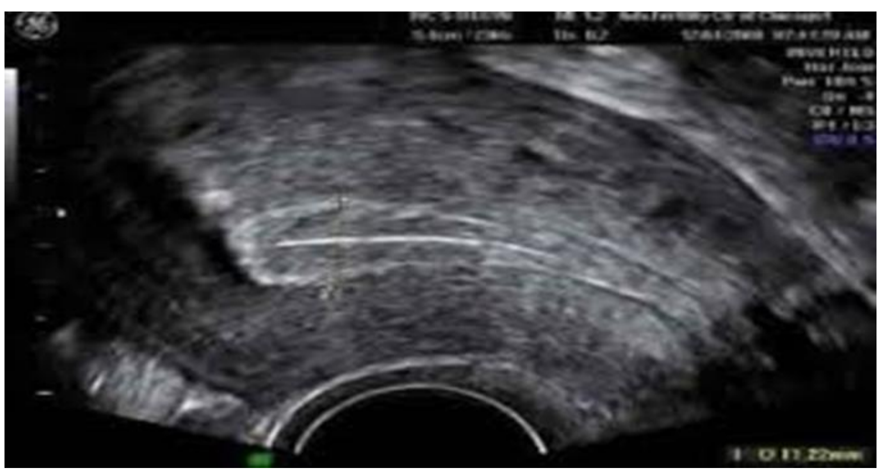

Figure (3): shows endometrial thickening

3. Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft Office 2010. The data was characterized using descriptive statistics, including measures of frequency, mean, and standard errors. The groups were compared using an independent sample t-test to assess the differences between the two groups. The chi-square test was used to compare non-continuous variables or percentages. An unpaired sample t-test was used to compare continuous variables in two periods, namely before and after. The results were considered statistically significant if the value was equal to or less than 0.05.

4. Results

There were no significant differences between pregnant and non-pregnant females regarding age grouping (< or ≥ 30 years) (p=0.495), body mass indices ranking (average weight, overweight and obese) (p=0.073), duration of infertility grouping (< or ≥ five years) (p=0.176), type of infertility (Primary or secondary) (p=0.475) and causes of infertility (Male, female, unexplained and combined causes) (p=0.737) as presented in Table 4.1

Comparison of Antral follicle count (AFC) before and after vitamin B12 supplements

There was a significantly higher Antral follicle count (AFC) (6.54 ± 0.55 vs.7.72 ± 0.26; p <0.001) among females who received vitamin B12 supplements shown in Table 4.2

Correlation between serum vitamin B12 with Antral follicle count (AFC)

There was also no significant correlation between serum vitamin B12 and Antral follicle count (AFC) (r=0.009 & p =0.948), as presented in Table 4.3.

***Table (2)*: Comparisons between pregnant and non-pregnant females with vitamin B12 supplements according to age, BMI, duration, and type of infertility grouping**

Parameters		**Positive pregnancy**	**Negative pregnancy**	***p-value***
Age grouping	**< 30 years**	10 (71.4 %)	22 (61.1 %)	0.495 NS
	≥ 30 years	4 (28.6 %)	14 (38.9 %)	
BMI ranking n. (%)	**Normal weight**	5 (35.7 %)	7 (19.4 %)	0.073 NS
	Overweight	9 (64.3 %)	19 (52.8 %)	
	Obese	0 (0.0 %)	10 (27.8 %)	
Duration of infertility(years) n (%)	**< 5 years**	6 (42.9 %)	23 (63.9 %)	0.176 NS
	≥ 5 years	8 (57.1 %)	13 (36.1 %)	
Type of infertility n.(%)	**Primary**	7 (50.0 %)	14 (38.9 %)	0.475 NS
	Secondary	7 (50.0 %)	22 (61.1 %)	
Cause of infertility n. (%)	**Male causes**	4 (28.6 %)	15 (41.7 %)	0.575 NS
	Female causes	3 (21.4 %)	7 (19.4 %)	
	Unexplained	0 (0.0 %)	2 (5.6 %)	
	Combined causes	7 (50.0 %)	12 (33.3 %)	

SE: Standard error; S: Significant (p≤0.05); NS: Not significant (p>0.05)

Table (2): Comparison of Antral follicle count (AFC) before and after B12 supplements

Parameters (Mean±SE)	**Before B12 supplement**	**After B12 supplement**	***p-value***

Antral follicle count (AFC)	6.54 ± 0.55	7.72 ± 0.26	< 0.001 Ƥ S

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

Table (3): Correlation between serum B12 and antral follicle count (AFC)

	Pearson's correlation coefficient (r)	**p-value**
Correlation between serum B12 and antral follicle count (AFC)	0.009	0.948 NS

NS: Not significant ($p > 0.05$)

5. Discussion

Comparisons between pregnant and non-pregnant females with vitamin B12 supplements according to age, BMI, duration, and type of infertility grouping

There were effects on infertility. We show lower levels of vitamin B12 in infertile couples than in pregnant but no significant differences between them in our study regarding age grouping (< or ≥ 30 years) (p=0.495), body mass indices ranking (average weight, overweight and obese) (p=0.073), duration of infertility grouping (< or ≥ five years) (p=0.176), type of infertility (Primary or secondary) (p=0.475) and causes of infertility (Male, female, unexplained and combined causes. Our results agree with previous research(23), including 726 patients divided according to pregnancy or not, age, primary or secondary infertility, and body mass index. Revealed that there were no substantial differences in the amounts of vitamin B12 between the pregnant and infertile groups and that the levels of vitamin B12 were considerably lower in the infertile patient group but disagreed in the body mass index (BMI)of the infertile group was significantly higher than that of the pregnant group. Little research hasn't been done on how age, BMI, and the length of infertility affect B12 levels while supplementing.

Age-related declines in vitamin B12 absorption may impact both pregnant and non-pregnant women. Their study shows a higher Antral follicle count (AFC) in females who received vitamin B12 supplements because vitamin B12 decreases the level of homocysteine, which is essential in increasing oxidative stress and accumulation of ROS that impair cell division, oocyte maturation, fertilization, embryo development, and lead to cell death; nevertheless, homocysteine reduction improved oocyte maturity and quality(22) (23). Furthermore, B12 keeps folate metabolism regular, which is necessary for cell division, rapid cell proliferating, placental growth, and fetal tissue formation. The study disagrees with this result and shows no relation between all types of vitamin B and intrafollicular count (AFC) except folate. (26)

There was also no significant correlation between serum vitamin B12 and antral follicle count. No previous study has correlated the relationship between them, but because of the effect of vitamin B12 on ROS.

It is also known that oxidative stress, which happens when the body's antioxidant defense system is compromised and pro-oxidant chemicals like reactive oxygen species (ROS) are out of equilibrium, can negatively impact fertility. While reactive oxygen species (ROS) are essential for regular cell functions, excessive production of ROS can hinder physiological reactions in women, including ovulation, luteolysis, follicle atresia, and oocyte maturation. This can result in infertility and conditions that can further impair fertility, such as endometriosis and polycystic ovary syndrome (PCOS)

Acknowledgement

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq

Funding

This work received no funding .

Author Contribution

Mouroge Khalaf Atea , performed the study, Laith Amer Al-Anbari supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure .

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