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MASTER'S THESIS

Specialty: Applied Biochemistry

Presented and supported by:
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The: 2025

Discovering the link between vitamin B12 deficiency and infertility.

Jury :

Title	First jury member	Rank	University of Biskra	President
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Dedication

To the one who has always been my source of light in every dark path,

the meaning of patience in every moment of weakness...

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the importance of striving and chasing dreams...

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To my pillars of strength, my dear siblings:

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You are the joy that lights up my days, and the unwavering support that lifts me when I fall.

And to the one who had the greatest impact on my academic journey,

my esteemed supervisor **Ms. Warda Kherour**, a true example of dedication, sincerity, and scientific integrity.

To all of you...

I dedicate the fruit of my effort, the result of my sleepless nights,

as a token of love, loyalty, and endless gratitude.

Adila nadjah

Dedication

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My esteemed supervisor, Ms. Warda Kherour, is a true example of dedication, sincerity, and scientific integrity.

To all of you...

I dedicate the fruit of my effort, the result of my sleepless nights,

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Ourchane Sara

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Liste of Abriviation

Vit-B12 :Vitamin B12

25(OH) D: 25-Hydroxyvitamin D

MTHFR: Methylenetetrahydrofolate reductase

MMA: Methylmalonic Acid

HoloTC :Holotranscobalamin

FSH**:** Follicle-Stimulating Hormone

LH : Luteinizing Hormone

E2 :Estradiol

AMH: Anti-Müllerian Hormone

SHBG: Sex Hormone-Binding Globulin

MDA :Malondialdehyde

GSH: Glutathione

SOD: Superoxide Dismutase

TAC: Total Antioxidant Capacity

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

ART: Assisted Reproductive Technology

WHO: World Health Organization

IVF: In Vitro Fertilization

ICSI : Intracytoplasmic Sperm Injection

BMI : Body Mass Index

DNA : Deoxyribonucleic Acid

DOI : Digital Object Identifier

NTDs: Neural Tube Defects

THF: Tetrahydrofolate

5-MTHF: 5-Methyltetrahydrofolate

SAM*:* S-Adenosyl Methionine

CoA *:* Coenzyme A CoA

NF-κB **:**Nuclear Factor kappa-light-chain-enhancer of activated B cells**.**

Myc: Myc proto-oncogene

Fos *:*Fos proto-oncogene

Introduction

Introduction

Infertility is a condition that affects approximately one in five to six couples of reproductive age. In the context of reproductive health, infertility refers to a deficiency that does not compromise an individual's physical integrity and is defined as the inability to achieve pregnancy after a reasonable period of unprotected intercourse without the use of contraceptive methods **(Brugo-Olmedo *et al*., 2001).**

Many women and men experience psychological distress as a result of failed attempts to conceive. Typically, infertility is diagnosed after 12 months of regular, unprotected intercourse if the woman is 34 years old or younger, or after 6 months if she is 35 or older, or if there is a history of recurrent miscarriage **(Cousineau & Domar, 2007)**. For many couples, infertility is often presumed to be an issue rooted in the female partner, a perception reinforced by myths and narratives passed down over thousands of years.

In recent years, studies exploring the relationship between diet and human fertility have expanded significantly, leading to the identification of certain clear patterns **(Gaskins & Chavarro, 2018).**

Vitamin B12, also known as cobalamin, exists in several forms, including cyano-, methyl-, deoxyadenosyl-, and hydroxycobalamin. Cyanocobalamin, commonly used in dietary supplements, is found in only trace amounts in food. Vitamin B12 deficiency was first described in 1849 and was believed to be fatal until 1926, when it was discovered that a diet rich in B12 could slow disease progression **(O'Leary & Samman, 2010).**

This vitamin is essential for various biological functions such as DNA synthesis and repair, as well as cell division. Therefore, its deficiency leads to the accumulation of methylmalonic acid and impaired DNA synthesis. Chronic deficiency may result in irregular ovulatory cycles, and in extreme cases, anovulation. If pregnancy does occur, B12 deficiency may cause alterations in egg development or lead to miscarriage **(Ibeh *et al*., 2019).**

The synthesis is divided into two main parts:

The first part is a literature review, itself divided into two chapters. The first chapter focuses on vitamin B12, while the second chapter provides general information about Infertility.

The second part is devoted to the analysis of the selected scientific studies. It is also divided into two chapters. The third chapter discusses the methodology used in the

selected studies, detailing the research question, the study objectives, and the methodological approach adopted. The fourth chapter presents the results derived from this synthesis, followed by their discussion and analysis.

Part 1

Bibliographic Synthesis

Chapter 01
Generalities about the vitamin

1.1. Vitamin B12 (Cobalamin)

1.1.1. Definition de la vitamine B12

Vitamin B12, also known as cobalamin, is a water-soluble vitamin that contains cobalt and serves as a cofactor for metabolically important enzymes. It is a general term for all forms of cobalamins active in humans, include cyanocobalamin, hydroxocobalamin, methylcobalamin, and 5-deoxyadenosylcobalamin **(Gherasim *et al*., 2013).** Structurally, it consists of cobalt as the central atom within a corrin ring that encloses the metal atom **(Fedosov, 2012). (Figure 1)**

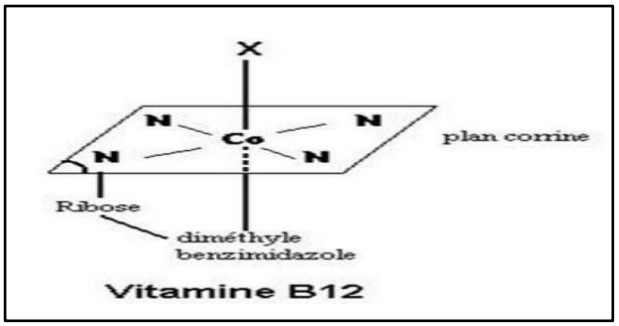

Figure 2. The chemical structure of vitamin B12 **(Watine *et al*., 2002).**

1.1.2. Food Sources and Bioavailability of Vitamin B12

Vitamin B12 is produced by bacteria and archaea and is naturally found only in animal-derived foods, such as meat, liver, fish, eggs, and dairy products **(Allen, 2008; Heyssel *et al*., 1966).** Although human gut bacteria can synthesize B12, the body cannot absorb it, making dietary intake essential **(Gille & Schmid, 2015)**.

There are no bioactive plant sources of B12. Some fortified foods, seaweed, and mushrooms contain B12 analogues, which are inactive in humans. However, some studies suggest that Japanese nori may help prevent B12 deficiency in vegans. Additionally, certain fermented and bacteria-contaminated foods (e.g., tempeh and Thai fish sauce) have been reported to contain vitamin B12 **(Stabler & Allen, 2004). (Table 1).**

Table 2. Vitamin B12 Content in Food Sources. **(Daine, 2017).**

Food Sources	Vitamin B12 Content (µg/100g)
Cooked liver (lamb, veal, or beef)	42.5 to 74
Raw oyster	24 to 39.5
Canned sardines	14
Cooked shrimp	5
Canned tuna	4
Cooked salmon	2 to 3
Grilled beef steak	2.7
Emmental, mozzarella, gouda, camembert	1 to 2.5
Hard-boiled egg	1.1
Chicken	0.5
Cow's milk	0.4
Plain yogurt	0.2
Goat's milk	0.1

1.1.3. Nutritional references (recommended dietary intakes)

The amount of vitamin B12 we need depends on our age and stage of life, as shown in the table below. (Table 3)

Table 4. Recommended daily intakes of vitamin B12 **(Daine, 2017).**

Categories	Nutritional Reference for Vitamin B12 (µg/day)
Children aged 1 to 3 years	0.8
Children aged 4 to 6 years	1.1
Children aged 7 to 9 years	1.4
Children aged 10 to 12 years	1.9
Adolescents aged 13 to 15 years	2.3
Adolescents over 16 years and adults	4.0
Pregnant women	2.6
Breastfeeding women	2.8

1.1.4. Metabolism and Absorption of Vitamin B12 in the Body

The main source of vitamin B12 is animal proteins. It is released in the stomach by pepsin and gastric acid, then binds to R-protein from saliva. In the duodenum, pancreatic enzymes release B12 from R-protein, allowing it to bind to intrinsic factor (IF) from gastric parietal cells

(Oh & Brown, 2003). The B12–IF complex is stable and binds to receptors in the terminal ileum for calcium-dependent absorption. After ~3–4 hours, B12 enters the bloodstream bound to transcobalamin II (holo TC-II) and is distributed to tissues like the liver and bone marrow, with the liver storing ~90% of total B12 **(Andrès *et al*., 2004).** B12 is secreted in bile and reabsorbed in the enterohepatic circulation, which also depends on IF—explaining the rapid deficiency seen in pernicious anemia **(Nutrients *et al*., 2000; Schjønsby, 1989). (Figure 3)**

Excretion occurs mainly via feces, which include unabsorbed B12, sloughed cells, and bacterial B12. Around 0.1% of body stores are lost daily **(Heyssel *et al*., 1966).** Excess B12, especially from injections, is excreted in urine once binding capacity is exceeded **(Schjønsby, 1989).**

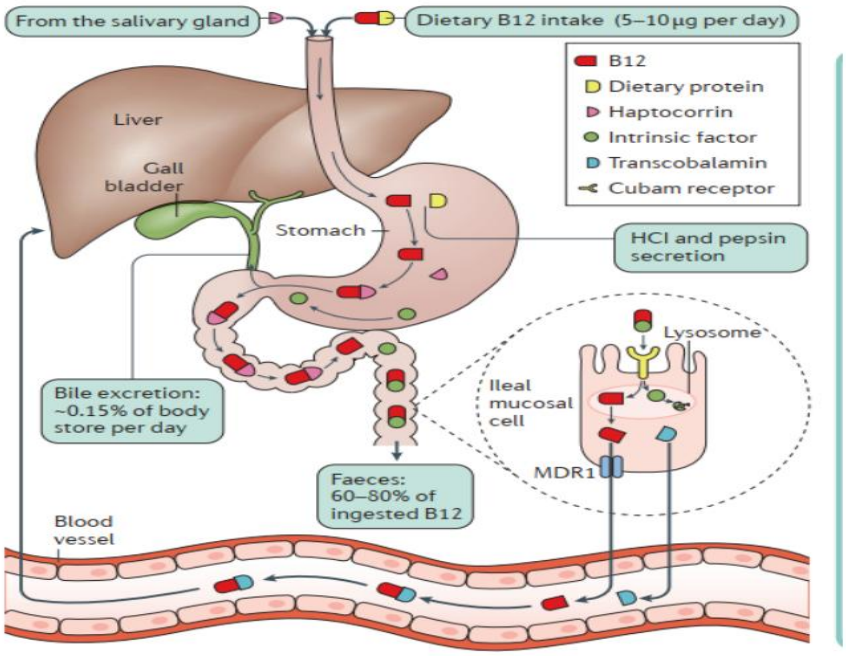

Figure 4. Absorption and Enterohepatic Pathway of Vitamin B12 **(Green *et al*., 2017).**

1.1.5. Intracellular Function of Vitamin B12

Within target cells, vitamin B12 is converted inside cells into cob (II)alamin, which is then transformed into either methylcobalamin or adenosylcobalamin, both involved in essential metabolic pathways **(Gherasim *et al*., 2013).**

A. Methionine Synthase Pathway

In the methionine synthase (MS) pathway, methylcobalamin donates a methyl group to homocysteine, forming methionine and cob (I) alamin. The latter regenerates methylcobalamin by accepting a methyl group from methyltetrahydrofolate, releasing tetrahydrofolate

(Gherasim *et al*., 2013; Chen *et al*., 1997). This reaction is catalyzed by the MS enzyme encoded by the CblG gene **(Leclerc *et al*., 1996).**

A vitamin B12 deficiency disrupts this process, trapping folate in its inactive form and raising homocysteine levels, which serve as a marker of cellular B12 deficiency. Similarly, folate deficiency can elevate homocysteine due to its role as a methyl donor.

This pathway explains clinical effects of B12 deficiency, such as megaloblastic anemia from impaired DNA synthesis and reduced SAM production, which is vital for methylation processes (**Obeid, 2013).**

B. Methylmalonyl-CoA Mutase Pathway

Adenosylcobalamin is synthesized in the mitochondria through the action of ATP-dependent cobalamin adenosyltransferase. It serves as a cofactor for MCM, which catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA, an intermediate of the Krebs cycle.

This radical-based reaction involves free radical formation and hydrogen migration. A B12 deficiency inhibits this conversion, resulting in the accumulation of methylmalonyl-CoA, which is hydrolyzed to methylmalonic acid (MMA) by methylmalonyl-CoA hydrolase (MCH)

Elevated MMA levels in plasma are used as a diagnostic marker to assess intracellular vitamin B12 status **(Gherasim *et al*., 2013). (Figure 5)**

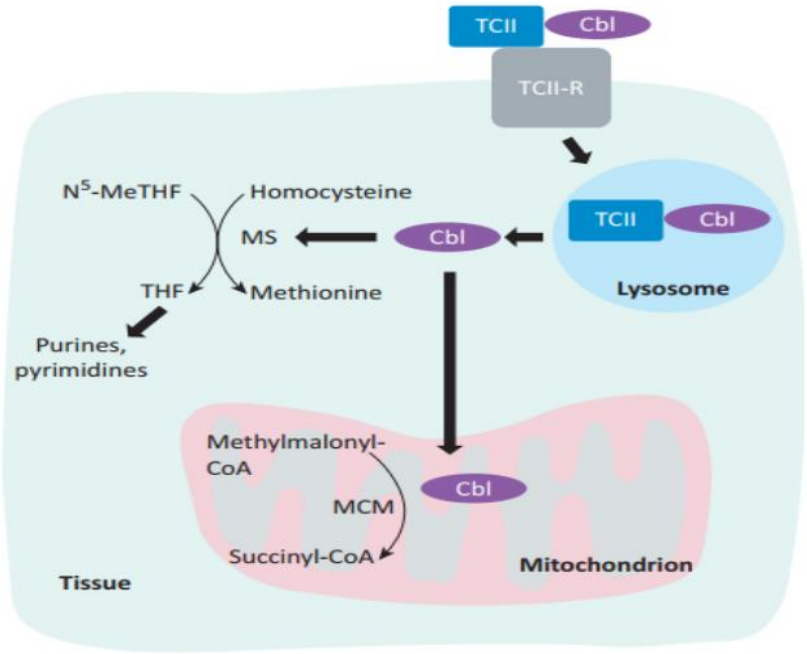

Figure 6. Intracellular Metabolism of Vitamin B12 **(Shipton & Thachil, 2015).**

1.1.6. The Physiological Role of Vitamin B12

- Methylation and Methionine Formation: Vitamin B12 acts as a cofactor in converting homocysteine to methionine, which forms SAM, a universal methyl donor for molecules like myelin and neurotransmitters **(Malouf *et al*., 2003).**
- Conversion of Methylmalonyl-CoA: B12 supports the conversion of methylmalonyl-CoA to succinyl-CoA in the Krebs cycle; deficiency causes elevated MMA and disrupts neuronal membrane lipid synthesis **(Manzanares & Hardy, 2010).**
- Neurotransmitter Synthesis: B12 is crucial for producing monoamines like serotonin and dopamine; its deficiency can lead to neurological disorders **(Bottiglieri *et al*., 2000).**
- Folate Recycling and DNA Synthesis: B12 converts 5-MTHF to THF, enabling DNA precursor synthesis; a deficiency causes folate trapping, reduced THF forms, and megaloblastic anemia **(Shane & Stokstad, 1985).**
- Potential Role in Gene Regulation: Vitamin B12 may aid in isomerizing D-leucine, influencing transcription factors such as NF-κB, Myc, and Fos, suggesting a potential oncogenic role **(Wheatley, 2006).**
- Energy Production: B12 facilitates the formation of succinyl-CoA, supporting cellular respiration and metabolic energy **(Manzanares & Hardy, 2010).**

1.7. Measurement of Plasma Vitamin B12

There is no gold standard for B12 assessment. It is transported by holoTC (active) and haptocorrin (inactive) proteins **(Green, 2011)**.

The standard test uses chemiluminescence to measure total plasma B12. It detects severe deficiency (<148 pmol/L) with high sensitivity (90–95%) and specificity (~80%), while subclinical deficiency (<220 pmol/L) has variable sensitivity (40–80%) **(Oberley & Yang, 2013).**

Results vary by lab, and conditions like HIV, folate deficiency, or pregnancy may cause false low readings **(Carmel, 2011).**

Chapter 02

Anatomical And Physiological Reminder of the Genital Apparatus

2.1. An anatomical and physiological reminder of the genital apparatus

2.1.1 Anatomy of the male genital apparatus

The human reproductive system comprises primary and secondary organs, with the male reproductive system primarily responsible for producing male gametes. These organs include the testicles, seminal vesicles, accessory glands, and penis **(Obukohwo *et al*., 2021).**

2.1.2 Testicles

The male gonads, paired testes, are located in the scrotum, maintaining a temperature 1°-3°C below the body's core for optimal sperm production. Each testis is a small, oval-shaped gland with a tunica albuginea membrane, dividing it into multiple lobules **(Patton & Thibodeau, 2015).**

2.1.3 Sperm Ducts

Sperm, derived from the Latin word "spermatozoan," are tiny, peculiar cells that participate in the reproductive process, resembling a seed. They have a tail and move independently, with nuclear chromosomes containing fetal traits acquired during fertilization**. (Patton & Thibodeau, 2015)**. The shape of sperm has always been the best indicator of male fertility **(Van Waart *et al*., 2001).**

2.1.4 Accessory Glands

Male accessory sex glands, including seminal vesicles, prostate, Cowper's glands, ampullar glands, little's glands, and Tyson's glands, are essential for the male reproductive system **(Singh, 2015).**

2.1.5 Penis

The penis, a sexual organ, deposits sperm into the vagina during intercourse, consisting of three separate erectile tissues, with the glans penis at its distal end **(Patton & Thibodeau, 2015).**

2.2 Anatomy of the female genital apparatus

The female reproductive system consists of internal and external genitalia, including the ovaries, fallopian tubes, uterus, vagina, and vulva, all located in the pelvic cavity **(Baillon, 2020)**.

2.2.1 Ovaries

Paired ovaries, weighing 3-6 grams, produce gametes and hormones, influenced by a woman's age and reproductive stage, supported by ligaments like the wide ligament **(Kleeman & Silva, 2007).**

2.2.2 Vagina

The vagina is a flexible, fibromuscular canal connecting external genitalia to the uterus, with three layers: an inner mucosal layer, a middle smooth muscle layer, and an outer adventitia **(Kelsey, 2023).**

2.2.3 Vulva

The vulva, comprising female external genitalia like the mound pubicus, labia majora and minor, clitoris, urethral opening, vaginal opening, and vestibular bulbs, protects pubic symphysis and reduces infection risk **(Lentz & Gershenson, 2012).**

2.2.4 Fallopian tubes

The fallopian tubes, also called the oviducts, are a pair of slender, muscular tubes that radiate outward to their apertures close to the ovaries from each corner of the uterus's body, about 10 cm apart **(Kleeman & Silva, 2007).**

2.2.5 Uterus

The urethra is a 3-5 cm long passageway from the bladder to the vestibule, lined by stratified transitional and squamous epithelium. In women, it passes through the external urethral sphincter, which must be relaxed for urination **(Kleeman & Silva, 2007).**

2.2.6 Ovarian Cycle

The ovarian cycle involves the development, maturation, and ovulation of ovarian follicles, affecting over 99% of oocytes and follicles that do not survive into adulthood **(Richards, 2018).**

2.2.7 Menstrual Cycle

Women's menstrual cycles are characterized by a fertile period that lasts from five days before ovulation, low fertility influenced by age and cycle length, and considerable variability in cycle duration (26–35 days) **(Mihm *et al*., 2011).**

2.3.1 Fecundity

Fecundity is a fundamental concept in reproductive biology and human fertility studies. It refers to the biological capacity of an individual or couple to reproduce, regardless of whether conception occurs **(Bongaarts, 1975).**

Fecundity in humans is influenced by genetic, hormonal, anatomical, and physiological factors, beginning with puberty and declining with age. Female fecundity peaks in the early 20s and declines after 35 due to oocyte reduction **(Broekmans *et al.*, 2009).**

Spermatogenesis, the process of sperm production in the testes, is primarily responsible for male fertility, which can be reduced by age-related changes in semen parameters **(Kidd *et al*., 2001).**

Environmental and lifestyle factors significantly influence fecundity, with exposure to toxins, heavy metals, pesticides, and endocrine-disrupting chemicals negatively impacting reproductive potential in both sexes **(Augood *et al*., 1998).**

Fecundity is measured by time to pregnancy (TTP), with high fecundity associated with shorter TTP. Reduced fecundity after 12 months of unprotected intercourse warrants further investigation **(Practice Committee of the American Society for Reproductive Medicine, 2013).**

2.3.2 Fecundability

Fecundability is the probability of conceiving in a single menstrual cycle of unprotected intercourse and reflects reproductive efficiency **(Weinberg *et al*., 1989).** Unlike fecundity, it provides a practical, probabilistic measure of how quickly a couple might conceive. The average fecundability rate in healthy couples is about 20–25% per cycle **(Joffe, 2000).**

It declines with age, especially after 35 in women due to decreased oocyte quality **(Dunson *et al.*, 2002)**, and is also affected by male age-related changes in sperm quality. Lifestyle factors such as smoking, alcohol, obesity, and stress reduce fecundability (**Augood *et al*., 1998; Rich-Edwards *et al*., 2002)**. Intercourse timing around ovulation increases chances of conception, while prior contraceptive use can have temporary effects **(Vessey *et al*., 1983).**

Fecundability is assessed in cohort studies using methods like Kaplan-Meier or survival models based on time to pregnancy **(Gnoth *et al*., 2003).** Clinically, it helps guide fertility counseling and public health monitoring.

2.3.3 General Factors Influencing Couples' Fertility

Fertility is influenced by biological and environmental factors, with alcohol, medications, and tobacco being major lifestyle factors that disrupt hormonal balance, damage reproductive organs, and interfere with gamete quality.

a) Alcohol

Alcohol disrupts reproductive hormones. In women, it causes anovulation and reduced ovarian reserve **(Muthusami & Chinnaswamy, 2005),** while heavy drinking lowers conception chances **(Tolstrup *et al*., 2003)**. In men, it damages sperm quality and lowers testosterone **(Emanuele & Emanuele, 2001)**.

b) Medications

Medications such as NSAIDs can block ovulation **(Akil *et al*., 1996)**, and chemotherapy drugs may cause ovarian failure **(Meirow *et al*., 2000)**. Hormonal drugs can raise prolactin, leading to infertility **(Molitch, 2005)**. In men, anabolic steroids and other drugs impair sperm production **(Turek & Master, 2004).**

c) Tobacco

Tobacco accelerates oocyte loss and disrupts hormones in women, increasing infertility risk **(Mattison & Thorgeirsson, 1978; Sharara & Seifer, 2001; Augood *et al*., 1998).** In men, it damages sperm and causes erectile issues **(Kunzle *et al*., 2003**), with reduced sperm quality and increased DNA damage **(Sharma *et al*., 2016).** Smoking also lowers ART success rates **(Stillman *et al.*, 1986).**

c) Conclusion

These factors reduce fertility, and couples should be counseled on their risks and consider lifestyle changes to improve reproductive outcomes.

2.3.4 Etiology of Female Infertility

Infertility, a condition resulting from unprotected sexual intercourse, can be caused by hormonal imbalances, ovulatory dysfunction, tubal factors, uterine abnormalities, cervical pathology, and endometriosis.

a) Cervical Causes

The cervix supports fertility by allowing sperm passage and producing mucus that aids sperm movement. Cervical factor infertility accounts for 5–10% of female infertility cases **(Berek & Novak, 2012).** Causes are either anatomical (e.g., cervical stenosis from surgery or trauma) or functional (e.g., thick or hostile cervical mucus due to hormonal imbalances or antisperm antibodies) **(Zegers-Hochschild *et al.*, 2009; Bronson & Alexander, 1988).** Diagnostic tools include postcoital testing, though its relevance has decreased **(Collins *et al*., 1984).** Treatments include cervical dilation or intrauterine insemination (IUI), which bypasses the cervix **(Speroff & Fritz, 2005).**

b) Endometriosis

Endometriosis affects about 10% of reproductive-age women and up to 50% of infertile women **(Giudice & Kao, 2004).** It involves endometrial-like tissue growing outside the uterus, causing inflammation, adhesions, and infertility through mechanisms such as anatomical distortion, ovarian dysfunction, peritoneal inflammation, immune system changes, and implantation failure **(Somigliana *et al.*, 2006; Dmowski *et al.*, 2001; Lessey, 2003)**. Diagnosis requires laparoscopy, with ultrasound or MRI aiding detection. Treatments vary by severity and fertility goals and include surgery **(Jacobson *et al.*, 2010),** medical therapy for pain, and IVF for severe or persistent cases **(Barnhart *et al.*, 2002).**

2.3.5 Etiology of Male Infertility

Male infertility, affecting 40-50% of cases, is primarily caused by varicocele and cryptorchidism, which directly affect testicular structure and spermatogenesis.

a) Varicocele

Varicocele is the abnormal enlargement of the pampiniform venous plexus, similar to varicose veins, and is the most common correctable cause of male infertility. It affects ~15% of men overall and up to 40% of infertile men **(Baazeem *et al.*, 2011)**, usually on the left side due to anatomical factors.

Pathophysiological mechanisms include

Testicular Hyperthermia – Varicoceles raise scrotal temperature, disrupting sperm production.

Hypoxia and Oxidative Stress – Poor venous drainage increases reactive oxygen species, leading to sperm DNA damage and reduced motility **(Agarwal *et al.*, 2006).**

Hormonal Imbalance – Varicocele may impair Leydig cell function and alter testosterone levels.

Reflux of Metabolites – The backflow of renal and adrenal toxins may harm testicular tissue **(Saypol, 1981).**

Diagnosis

Varicocele is primarily diagnosed through physical examination and confirmed by scrotal Doppler ultrasonography, which reveals dilated veins (greater than 2–3 mm) and retrograde blood flow during the Valsalva maneuver. It is classified into grades:

- Grade I: Palpable only during Valsalva.

- Grade II: Palpable without Valsalva.

- Grade III: Visible through the scrotal skin.

Part 2

Summary of the Selected Scientific Works

Chapitre 3

Methodology followed in the selected works

3.1. Study objectives

This study aims to evaluate the relationship between vitamin B12 deficiency and infertility in humans by analyzing available observational and interventional studies focusing on the effects of this deficiency on fertility in both men and women. The study will examine semen parameters (sperm count, motility, morphology) and ovulation/menstrual irregularities, as well as biochemical factors such as vitamin B12 and homocysteine levels. The study also aims to assess the effect of vitamin B12 supplementation on improving fertility and increasing the chances of conception and live birth.

3.2. Research Questions Addressed

The research questions for this systematic review were formulated based on recommendations from other group EPOC reports from the Cochrane Collaboration and the PICOT method (Population, Intervention, Comparator, Outcome, Study Type) **(Higgins *et al*., 2023):**

Question: Is vitamin B12 deficiency associated with an increased risk of infertility in humans?

3.3. Study Overview

To achieve the objectives of this study and address the targeted research questions, the recommendations from the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guide were followed to conduct this study rigorously and transparently **(Moher *et al*., 2009).**

3.4. Study Selection

3.4.1. Question

Is vitamin B12 deficiency linked with an increased risk of infertility?

3.4.1.1. Inclusion Criteria (Based on PICOT)

Studies are considered eligible for inclusion if they meet the following inclusion and exclusion criteria:

- **Population**: Adults of reproductive age (both males and females) diagnosed with or assessed for vitamin B12 deficiency and/or infertility.
- **Intervention (for intervention studies) :** Vitamin B12 supplementation.

- **Comparator:** Fertile controls or individuals with normal vitamin B12 levels (for observational studies); placebo or standard care (for intervention studies).
- **Outcomes :**
  - **Primary outcomes:** Infertility diagnosis (defined by established criteria), semen parameters (sperm count, motility, morphology), ovulation/menstrual irregularities, time to pregnancy, live birth rate.
  - **Secondary outcomes:** Serum vitamin B12 levels, homocysteine levels, other relevant biochemical markers.

- **Study type**: Observational studies (cohort, case-control, cross-sectional), interventional studies (RCTs).

3.4.1.2. Exclusion Criteria

- **Population:** Studies including participants with other conditions affecting fertility that are not related to vitamin B12 deficiency (such as cancer or congenital abnormalities).
- **Intervention:** Studies focusing solely on vitamin B12 supplementation without measuring its levels as a risk factor.
- **Comparator:** Studies that do not clearly define a comparison group.
- **Outcome:** Studies using unreliable or unvalidated diagnostic criteria for infertility.
- **Study Type:** Case reports, case series, and narrative reviews.

3.5. Study Selection Process.

The selection of relevant studies for this systematic review followed a process. rigorous and transparent in several stages, guided by PRISMA recommendations (Moher et *al*., 2010).

3.5.1 Identification and removal of duplicates.

From April 2024 to May 2024, an initial search in the PubMed and ScienceDirect databases Scopus allowed the identification of a set of potential studies. The management tool Bibliographic Zotero was then used to eliminate duplicates from this initial list.

3.5.2. Selection by titles and abstracts.

The researchers independently reviewed the titles and abstracts of the remaining studies using the tools Rayyan and Systematic Review Accelerator. This step allowed us to select potentially relevant articles for the systematic review based on their alignment with the research questions.

3.5.3. Selection by full read.

The full articles selected in the previous step were read and analyzed in depth to identify those that fully met the review's inclusion criteria and that contained sufficient data to answer the research questions.

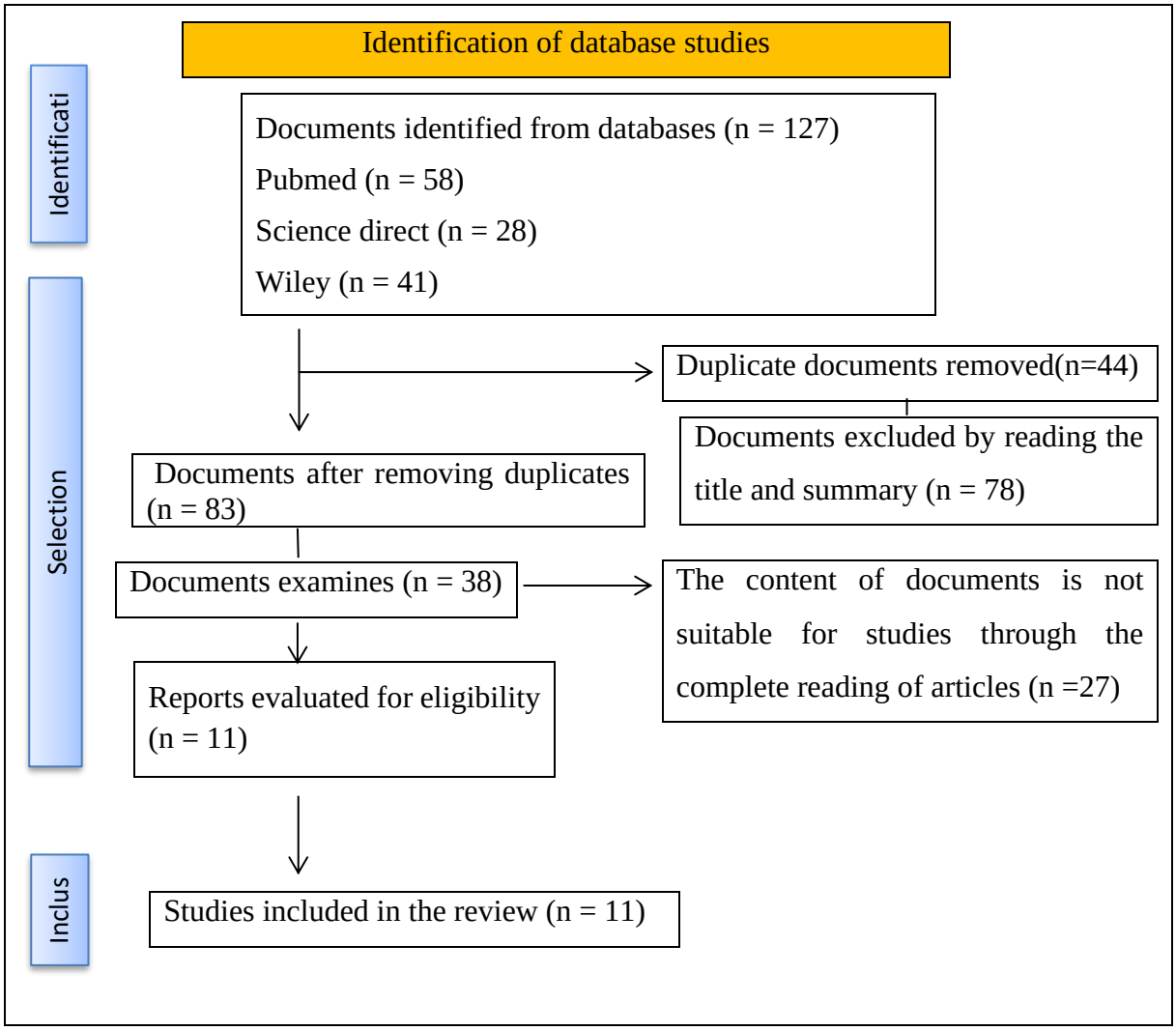

Figure 4. PRISMA diagram for the research question

3.6. Research strategy

Search strategies based on keywords and Boolean operators:

3.6.1. Keywords used to answer the first research question.

- Vitamin B12, cobalamin, cobalamin deficiency.
- Infertility, subfertility, semen quality, sperm parameters.
- Ovulation, menstrual cycle, pregnancy, conception, supplementation.

- biochemical markers") AND ("Vitamin B12" OR "Homocysteine" OR "Methylmalonic Acid (MMA)" OR "Holotranscobalamin (HoloTC)" OR "Follicle-stimulating hormone (FSH)" OR "Luteinizing hormone (LH)" OR "Estradiol (E2)") AND ("Progesterone" OR "Anti-Müllerian hormone (AMH)" OR" Testosterone" OR "Sex hormone-binding globulin (SHBG)" OR "Inhibin B" OR "Malondialdehyde (MDA)" OR «Glutathione (GSH)" OR "Superoxide dismutase (SOD)" OR "Total antioxidant capacity (TAC)").

A. Research paragraph on PubMed.

((“Infertility”[MeSH Terms] OR “subfertility”[Title/Abstract] OR “Semen”[MeSH Terms] OR “sperm count”[Title/Abstract] OR “Sperm Motility”[MeSH Terms] OR “sperm morphology”[Title/Abstract] OR “Ovulation”[MeSH Terms] OR “Menstrual Irregularities”[MeSH Terms] OR “time to pregnancy”[Title/Abstract] OR “Live Birth”[MeSH Terms] OR “vitamin B12”[MeSH Terms] OR “homocysteine”[MeSH Terms] OR “biochemical markers”[Title/Abstract]) AND (“Vitamin B12”[MeSH Terms] OR “Homocysteine”[MeSH Terms] OR “Methylmalonic Acid”[MeSH Terms] OR “Holotranscobalamin”[Title/Abstract] OR “Follicle Stimulating Hormone”[MeSH Terms] OR “Luteinizing Hormone”[MeSH Terms] OR “Estradiol”[MeSH Terms] OR “Progesterone”[MeSH Terms] OR “Anti-Mullerian Hormone”[MeSH Terms] OR “Testosterone”[MeSH Terms] OR “Sex Hormone-Binding Globulin”[MeSH Terms] OR “Inhibin B”[Title/Abstract] OR “Malondialdehyde”[Title/Abstract] OR “Glutathione”[MeSH Terms] OR “Superoxide Dismutase”[MeSH Terms] OR “Total Antioxidant Capacity”[Title/Abstract])) AND (“Placebos”[MeSH Terms] OR “Control Groups”[MeSH Terms] OR “placebo”[Title/Abstract] OR “control”[Title/Abstract])) AND (Clinical Trial[Publication Type] OR Observational Study[Publication Type]).

3.7. Data extraction

The data were independently extracted by us and a reviewer who is our supervisor using a standardized data extraction form (Sam Bunjak et al., 2017) and by the PRISMA guidelines Systematic Reviews and Meta-Analyses (Moher et al., 2010). Disagreements were resolved. by discussion or by involvement.

The general data are mentioned below, while the specific data for each research question are detailed in the Results and Discussion chapter.

Study characteristics: Author, Year of publication, Country, Study design, Duration of the study

Participants: Population studied, Sample size, Age group, Sex.

Interventions: Description of the intervention, Dosage, and duration.

Results: Measure(s) of the main evaluation criterion, Measure(s) of the secondary evaluation criteria.

Chapter 4

Results and Discussions

4.1. General Study Information

The attached table provides a summary of 11 scientific studies examining the relationship between vitamin B12 and human infertility. It includes basic information such as: the study title, researchers' names, year of publication, the name of the scientific journal, and the country or region where each study was conducted (**Table03).**

Table 5. General Study Information

N°	Study title	Authors	Year of publication	Journal name	Country or region of study
01	"To investigate the relationships between 25(OH)D & Vit-B12 levels and human fertility in healthy reproductive age women."	**(Shankar Bharti & Singh, 2023)**	May13, 2023	International Journal of Academic Medicine and Pharmacy (Int J Acad Med Pharm)	Kishanganj, Bihar, India
02	"Vitamin B 12 Is Associated with Higher Serum Testosterone Concentrations and Improved Androgenic Profiles Among Men with Infertility"	**(Rastegar Panah *et al*., 2024)**	June 26, 2024	not mentioned in the paper despite the presence of a DOI.	conduite in Canada, specifically at Mount Sinai Hospital in Toronto
03	"Vitamin B 12 and Semen Quality"	**(Banihani, 2017)**	June 9,2017	Biomolecules	Jordan (Jordan University of Science and Technology, Irbid)
04	"Use of multivitamins, intake of B vitamins and risk of ovulatory infertility."	**(Chavarro *et al*., 2008)**	September 1, 2008	"Fertility and Sterility," as indicated in the manuscript citation	Boston

05	"The effect of MTHFR gene polymorphisms and B vitamin intake on male infertility and sperm parameters."	**(Najafipour *et al*., 2017)**	February 4, 2017	Andrology	Iran (Qazvin University of Medical Sciences, Tehran-based institutions)
06	"Evaluation of Vitamin B12, Folate, Haematological Parameters and Some Reproductive Hormones in Subjects Attending Fertility Centres in Port Harcourt "	**(Isomah *et al*., 2021)**	August 6, 2021	European Journal of Medical and Health Sciences	Port Harcourt, Nigeria
07	"Study of Correlation between Serum Vitamin B12 Level and Aberrant DNA Methylation in Infertile Males"	**(Article, 2024)**	June 26, 2024	"Indian Journal of Clinical Biochemistry"	India
08	" Evaluation of Serum Vitamin B12 and Vitamin D Levels in Infertile Males with Suboptimal Semen Parameters – A Pilot Study from Eastern India"	**(Santra *et al*., 2022)**	2022	Journal of Clinical and Diagnostic Research (JCDR)	Eastern India (Kolkata, West Bengal, India)
09	"The relationship of sperm DNA integrity with serum vitamin levels (folate and cobalamin) and food consumption in infertile men"	**(Boushaba *et al*., 2023)**	2023	Clinical and Experimental Reproductive Medicine (CERM)	North African
10	"Correlation between serum vitamin B12, vitamin D, and suboptimal semen parameters in male infertility: A hospital-based cross-sectional study"	**(Kshatri *et al*., 2022)**	2022	amily Medicine and Primary Care.	north India

11	"Folate and vitamin B_{12} in idiopathic male infertility"	**(Murphy *et al.*, 2011)**	August 22, 2011,	Asian Journal of Andrology	Sweden, specifically in the cities of Lund and Malmo

4.1.1 Studies Involving Women (2 Studies)

Evidence from two studies focusing on women suggests a strong association between vitamin B12 levels and female reproductive health.

The first study **(Shankar Bharti & Singh, 2023)** showed a positive correlation between adequate or high levels of vitamin B12 and increased fertility during reproductive age. This likely reflects the vitamin's role in supporting hormonal balance and normal ovulatory cycles.

The second study **(Chavarro *et al.*, 2008)** found that adequate intake of B vitamins — including B12 — was associated with a reduced risk of ovulation-related infertility.

Biochemically, it is known that vitamin B12 acts as a cofactor in the conversion of homocysteine to methionine, a process that plays a crucial role in DNA synthesis and early embryonic cell differentiation. Therefore, low B12 levels may negatively affect the ovarian environment and hinder proper oocyte maturation.

4.1.2. Studies Involving Men (9 Studies)

The majority of available evidence comes from nine studies focused on men, with findings largely consistent in highlighting the importance of B12 in maintaining male fertility through various mechanisms:

4.1.2.1 Semen Quality

Several studies**(Banihani, 2017; Rastegar Panah *et al.*, 2024; Santra *et al.*, 2022)** demonstrated a link between B12 deficiency and decreased sperm count, motility, morphology, and volume. One study **(Najafipour *et al.*, 2017)** also noted a synergistic effect between B12 and genetic mutations like MTHFR, which affect folate metabolism and semen composition, thus influencing fertility.

4.1.2.2 Hormonal Effects

Study 6 **(Isomah *et al.*, 2021)** showed that B12 deficiency was associated with alterations in hormones such as FSH and LH, and hematologic factors affecting sperm production.

A Canadian study**(Rastegar Panah *et al.*, 2024)** found that men with B12 deficiency had lower testosterone levels and an imbalanced hormonal profile, suggesting a potential direct effect of B12 on regulating the hypothalamic-pituitary-gonadal (HPG) axis.

4.1.2.3 Genetic Integrity of Sperm

Two studies **(Boushaba *et al.*, 2023; Rastegar Panah *et al.*, 2024)** confirmed that low B12 levels are associated with increased epigenetic changes such as DNA hypermethylation in sperm, which may impair fertilization potential and pregnancy maintenance, or contribute to unexplained infertility.

4.1.2.4 Unexplained Infertility

A Swedish study**(Murphy *et al.*, 2011)** reported that B12 deficiency was common among men diagnosed with unexplained infertility, supporting the possibility that this deficiency could be an underlying factor in such cases.

4.1.3. Strengths and Limitations of the Available Literature

4.1.3.1. Strengths of the Review

Noticeable geographic diversity (India, Canada, Iran, Nigeria, Sweden, etc.), adding generalizability to the results.

Comprehensive coverage of both genders, expanding clinical applicability.

Multidimensional mechanisms explored — hormonal, genetic, and epigenetic — such as B12 effects on genes, testosterone, and sperm quality.

Most studies are recent (up to 2024).

4.1.3.2. Limitations and Observations

Methodological variation: most studies are observational, cross-sectional, or experimental, limiting the ability to establish causality.

Sample sizes varied; some studies were preliminary or small-scale with inconsistent measurement methods.

A lack of experimental (interventional) studies.

4.1.4. Clinical and Future Recommendations

These findings suggest that B12 should be considered a biological marker in routine fertility assessments for both men and women. B12 supplementation may be particularly beneficial for individuals with plant-based diets or gastrointestinal conditions that impair vitamin absorption.

From a research perspective, there is a pressing need for well-controlled interventional studies to evaluate the effectiveness of B12 supplementation in improving fertility outcomes and to determine optimal dosing, especially in cases of unexplained infertility.

4.2.Study Characteristics

This table provides a systematic summary of the characteristics of the scientific studies (11 studies). The aim of the table is to present a comprehensive and comparative overview of these studies in terms of design, methodology, and geographical and temporal context, which helps in understanding their research backgrounds and assessing the degree of their homogeneity or variability **(Table04).**

Table 6. Study Characteristics

N°	Study design	Study objective(s)	Duration of follow-up	Sample size	Setting
01	A cross-sectional study was conducted in the Department of Biochemistry at MGM Medical College and LSK Hospital in Kishanganj, Bihar. It included 100 reproductive-aged women between 18 and 25 years old. Blood samples were collected to analyze various hormone levels, including 25(OH)D, FSH, LH, and estradiol (E2).	The study aims to explore the relationship between vitamin D [25(OH)D], vitamin B12, and fertility in women of reproductive age. It focuses on the association between vitamin D and reproductive hormones such as testosterone and LH, as well as its impact on androgen levels and fertility rates in women with polycystic ovary syndrome (PCOS).	Not applicable	The study enrolled 100 reproductive aged women aged between 18 to 25 years . Participants were divided into two groups based on AMH levels. Group-A consisted of 40 women with high AMH levels, while Group-B had 60 women . .	The study was conducted at MGM Medical College and LSK Hospital, Kishanganj, Bihar
02	Cross-sectional study the paper recommends conducting randomized controlled trials to determine the optimal dose of vitamin B12 for patients with sickle cell disease, with	To examine the relationship between serum vitamin B12 and male reproductive hormones (including luteinizing hormone, follicular-stimulating hormone, total testosterone,	Not applicable	Total: 303 men with infertility (No division into groups mentioned beyond tertiles of B12 levels for analysis)	Mount Sinai Hospital, Toronto, Canada

	a focus on women in future studies. It also highlights the importance of multicenter studies and points to a comprehensive review of previous research on the effects of B12 on reproductive health in individuals with the disease.	estradiol, and prolactin) among men with infertility..			
03	The overall study design includes clinical, in vivo, and in vitro studies to evaluate the effect of vitamin B12 on semen quality. Ongoing clinical studies rely on bioanalytical methods such as flow cytometry to determine vitamin B12 concentrations. The research highlights the need for further clinical studies to confirm the potential benefits of this vitamin.	The study aims to investigate the effect of vitamin B12 on semen quality and reproductive organ function, and to explore the relationship between vitamin B12, homocysteine toxicity, and oxidative damage to sperm. It also includes clinical studies aiming to standardize vitamin B12 concentrations in human spermatozoa.	The study was conducted over a period of six months.	The study included 70 men who were randomly selected.	The study was conducted at the Department of Biochemistry, Jordan University of Science and Technology, Irbid, Jordan.
04	The study design is a prospective cohort study, in which a group of pre-menopausal married women attempting to conceive was followed from 1991 to 1999. The aim was to examine the relationship	/	8 years, from 1991 to 1999	/	/

	between diet, lifestyle, and chronic diseases.				
05	Interventional study (participants were given vitamin B9 and B12 for 6 months)	To investigate the effect of vitamin B9 and B12 intake on semen parameters and fertility in men with MTHFR gene polymorphisms.	6 months	The study included 70 volunteers in each group, 85 men with normal spermatogenesis as control samples, and sperm samples were collected from several oligospermic men. Serum samples from 60 participants were also analyzed to determine vitamin concentrations.	Isfahan Fertility and Infertility Center – Isfahan University of Medical Sciences, Isfahan, Iran.
06	The study employed a case-control and comparative design, utilizing a random convenient sampling method	The study aimed to assess vital parameters in infertility cases among healthy individuals aged 18 to 44 years. It compared haematological parameters and reproductive hormones between infertile and fertile groups, and also evaluated the significance of vitamin B12 levels in relation to infertility.	Not applicable	The study included 200 participants (100 females and 100 males) aged between 18 and 44 years. Each of the male and female groups consisted of 50 infertile individuals and 50 fertile individuals as the control group.	The study was conducted at Rivers State University Teaching Hospital and Save a Life Mission Hospital in Port Harcourt, Nigeria. A total of 200 participants were selected from fertility clinics, with ages ranging from 18 to 44 years.
07	The study employed a cross-sectional design to assess the correlation between serum vitamin B12 levels and DNA methylation in infertile males	The study aimed to investigate the correlation between serum vitamin B12 levels and aberrant DNA methylation in infertile male patients	Not applicable	The study consisted of 17 oligozoospermic infertile men as the case group and 10 healthy normozoospermic men	The study was conducted at the Department of Endocrinology and Metabolism, Institute of Medical Sciences, Banaras Hindu University

				as the control group, totaling 27 participants	(BHU), Varanasi, Uttar Pradesh, India.
08	Cross-sectional study	The study aimed to assess the association between serum vitamin B12 and vitamin D levels with semen parameters in infertile males from Eastern India	Not applicable	A pilot study involving 52 male subjects	Conducted at a tertiary care hospital in Kolkata, India
09	The study employed a cross-sectional design to assess infertility in men	The study aimed to investigate the relationship between food consumption and blood concentrations of vitamins B9 and B12 in infertile men It also explored the correlation of vitamin concentrations with semen standard parameters (SSP) and sperm DNA fragmentation (SDF)	Not applicable	The total sample size of the study was 29 infertile men Approximately 64% of the participants suffered from primary infertility	The study was conducted in a clinical setting at Ibn Rushd Laboratory of Medical Analysis within a North African population
10	The study employed a cross-sectional design conducted over two years from June 2021 to May 2023	The study aimed to examine serum levels of vitamin B12 and vitamin D in infertile males, with the goal of providing insights for targeted interventions to improve male reproductive health. It addressed the existing knowledge gap regarding the relationship between nutritional status and male fertility.	the study was conducted over a period of two years, from June 2021 to May 2023.	The total sample size of the study was 73 infertile males aged 20-40 years	The study was conducted at a tertiary health care centre in north India participants were recruited from the fertility clinic within the health care centre

11	The study design employed was a case-control analysis, comparing infertile men (cases) with fertile controls. Participants responded to a questionnaire to ascertain reproductive, medical, and dietary history, and provided blood and semen samples for analysis.	The study aimed to investigate the association between blood concentrations of folate, vitamin B12, and total homocysteine (tHcy) with male infertility.	Not applicable	The total sample size included 153 infertile men and 200 fertile men	The study was conducted in prenatal care clinics located in Lund and Malmo, focusing on fertile men and their partners

4.2.1. Study Design

The studies varied in design, reflecting a multi-faceted interest in the topic:

- **07** Cross-sectional studies **(Boushaba *et al.*, 2023; Rastegar Panah *et al.*, 2024; Santra *et al.*, 2022; Shankar Bharti & Singh, 2023):** Focused on the relationship between B12 levels and reproductive status at a single point in time. These studies are useful for identifying associations but do not establish causality.
- **02** Case-control studies **(Isomah *et al.*, 2021; Murphy *et al.*, 2011)**: Represent a stronger design than cross-sectional studies, as they compared groups of infertile individuals with fertile ones, helping to identify and understand differences in biological markers, including vitamin B12.
- **01** Interventional study **(Najafipour *et al.*, 2017):** This is the strongest in terms of scientific evidence. Participants received B12 supplements, and changes in semen quality were monitored. This study confirmed the positive effect of vitamin B12 on fertility.

- **01** Cohort study**(Chavarro *et al*., 2008)**: The only study that followed a group of women over 8 years, allowing for the assessment of whether low B12 levels may later contribute to reproductive problems.
- **01**Comprehensive study (Clinical + in vivo + in vitro) – **{Formatting Citation}:**This integrative study combined laboratory experiments with clinical samples to understand the biological mechanisms linking B12 with sperm quality and health.
-
  - The diversity in study designs enriches the findings and is considered a strength of this review. However, it also makes it challenging to unify conclusions. There is a need for more long-term experimental studies.

4.2.2. Study Objective

The common goal across all studies was to explore the relationship between vitamin B12 deficiency and infertility, but from different perspectives:

Studies **(Isomah *et al*., 2021; Rastegar Panah *et al*., 2024; Shankar Bharti & Singh, 2023)** focused on the relationship between B12 and sex hormones such as testosterone, LH, FSH, and estradiol, highlighting the hormonal role of B12.

Studies **(Banihani, 2017; Boushaba *et al*., 2023; Kshatri *et al*., 2022; Murphy *et al*., 2011; Najafipour *et al*., 2017; Santra *et al*., 2022)** examined seminal parameters (e.g., sperm count, motility, morphology, DNA fragmentation), directly linking B12 deficiency to semen quality and characteristics.

Studies **(Article, 2024; Najafipour *et al*., 2017)** concentrated on genetic and molecular mechanisms such as MTHFR polymorphisms and DNA methylation disorders, presenting B12 not just as a nutrient but also as a genetic regulator.

Study **(Chavarro *et al*., 2008)** focused on nutritional and lifestyle aspects as factors influencing fertility, including vitamin B12.

- The diversity of objectives reflects the comprehensiveness of the research but highlights the need for standardized outcome measures and broader focus on female populations.

4.2.3. Duration of Follow-up

Most studies were cross-sectional or instantaneous, and therefore lacked a follow-up period. One study**(Chavarro *et al*., 2008)** extended over 8 years, the only one allowing long-term tracking of changes.

One interventional study **(Najafipour *et al*., 2017)** lasted 6 months, which was sufficient to observe improvements in semen parameters and monitor the effect of dietary supplementation.

Study 10 was conducted over two years, offering more stable and reliable results.

4.2.4 Sample Size

Sample sizes ranged from only 27 participants **(Article, 2024)** to over 300 participants **(Rastegar Panah *et al*., 2024)**.

Studies **(Isomah *et al*., 2021; Murphy *et al*., 2011; Rastegar Panah *et al*., 2024)** included large and balanced samples in terms of gender and between cases and controls, enhancing the statistical power of the findings.

Some studies had limited sample sizes, such as Studies **(Article, 2024; Boushaba *et al*., 2023; Santra *et al*., 2022)** weakening the generalizability of their results.

- Sample size is a key factor in the credibility of findings. Therefore, it's essential to focus on multi-center studies with larger participant groups.

4.2.5. Study Setting

The studies were distributed across India (multiple studies), Canada, Jordan, Iran, Nigeria, and North Africa. This indicates international interest in the topic, but also highlights the limited research conducted in Europe and Latin America. Most of the studies were carried out in medical or university fertility centers, which enhances the credibility of the data collection.

- The geographic diversity is positive, but there is a need for multi-center studies to represent various ethnicities and dietary environments.

4.3. Population Characteristics

The table presents the population characteristics of participants in the 11 studies included in the analysis. It summarizes information related to the target group in each study, with the aim of clarifying the participants' backgrounds and highlighting the similarities or differences across the studies **(Table05).**

Table 7. Population Characteristics

N°	**Population type**	**Inclusion and exclusion criteria**	**Age**	**Sex**	**Ethnicity/race**	**Health status**
1	The study focuses on a specific population type, which consists of **healthy reproductive-aged women**	**Inclusion Criteria :**Age, Health Status, AMH Levels**Exclusion Criteria :**Hypogonadism ,Under lying Health Conditions	The study included women aged between **18 to 25 years**	specif ically focus es on **wome n**	The research specifically mentions that the findings are based on reproductive-age **Caucasian women**	**Group A:**Women with high levels of AMH, often with polycystic ovaries. **GroupB:**Women with normal levels of AMH.
2	The study focused on a cohort of **832 males** experiencing infertility	**Inclusion Criteria:** Males experiencing infertility were recruited from the Murray KofflerUrologic **Exclusion Criteria:** Individuals who had undergonepostoperativeprocedur es or had physicalimpairmentsleading to infertility were excluded, Those with recent use of fertility-relatedmedication, vasectomy, testicular cancer	between **18 to 25 years**	specif ically focus es on **males**	The majority of participants in the study identified as Caucasian Otherethnicities included Asian ,unspecified African-Canadian Middle-Eastern Indo-Canadian and Hispanic.	Changes in vitB12 levels (high and low) Changes in reproductive hormone levels (high and low) Body mass differences: 48.2% were overweight , and 24.1% were obese

		radiation therapy within the last four years				
	. Human Studies*Infertile men *Non-sterile men for comparison*Some studies have included **men with varicocele**	Focus only on studies that**examine Vitamin B12 × Semen Quality**directly and in detail, and excludeeverythingthat is irrelevant, incomplete, or unclear	**Adult human studies**(usually between 20–50 years)	**Males only** because the subject of the study focuses	**Indians** (Indian population **Japanese** **Spaniards** and otherdiverse nationalities	Men with fertility problems Especially cases like: **Oligozoospermia** Idiopathic infertility **Varicocele** Normozoospermia (normal men for comparison)
4	**Study** 1 (Vitamin D, B12 & Fertility in Indian Women) :The study included 100 healthy women **Study2** (**Multivitamins, B-Vitamins & Ovulatory Infertility):** This study was based , a large	**Study 1 – Inclusion Criteria:** Women aged 18–25 years, In reproductive age with no chronic diseases, Classified as clinicallyhealthy, Subdivided based on AMH levels . **Exclusion Criteria:** Anywoman with chronic illness or systemic disease **Study2 : Inclusion Criteria:**	Study1 : Participants were **reproductive-aged women between 18 and 25 years**. Study2 : Participants were married, premenopausal women	Both studies exclusivelyinvolved**female participants**, as the research focused on female reproductive health	**Study 1 :** The participants were **Indian** **Study2 :** The majority of participants were**White American women**	**Study1 :**Althoughconsidered healthy, manyexhibitedsubclinical hormonal imbalances and nutritional deficiencies, particularly in vitamin D and B12 **Study2 :** This study included married, premenopausal women with no prior history of infertility.

	prospective cohort of 18,555 married, premenopausal women in the United States	Married, premenopausal women aged 24–42 years, articipants in the Nurses' Health Study II, No history of infertility at baseline. **Exclusion Criteria:** Women with a history of infertility prior to the study, Those who had undergonesterilizationprocedures ,Postmenopausalwomen,Diabetic women	aged 24 to 42 years			
5	**Control group**: 85 men with normal semen parameters **Infertile groups**: 280 men divided into 70 men per group (asthenospermic, oligospermic, severely oligospermic, and azoospermic)	**Inclusion Criteria:** Men with normal spermatogenesis, Infertile groups, Asthenospermic,Oligospermic,Severeoligospermic,Azoospermic **Exclusion Criteria:** Cysticfibrosis,Klinefeltersyndrome,Varicocele,Chemotherapy,AZF gene microdeletions,Smokers,Alcohol consumers	**Control group (normal spermatogenesis): 34.0 ± 3.8 years** **Asthenospermic men (lowmotility): 29.1 ± 4.2 years** **Oligospermic men (low sperm count):**	The **sex** of all participants in the study is **male**.	The study participants were all from **Iran**,	the health status was generally**good**, aside from idiopathic infertility due to impaired**spermatogenesis**, which was the condition beingstudied

			32.0 ± 5.5 years **Severeoligospermic men: 34.6 ± 3.7 years** **Azoospermicmen (no sperm): 33.6 ± 5.4 years**			
6	**Sample Size**: 200 participants **100 infertile** individuals (50 males, 50 females) **100 fertile controls** (50 males, 50 females)	**Inclusion Criteria:** Adults aged 18 to 44 years Infertile group Individuals Fertile group (controls) Includes both**primary and secondary infertility** **Exclusion Criteria:** individuals not attending the specified fertility clinics Individuals not consenting to participate in the study Those not meeting the definitions of fertility or infertility	Age Range:**18 to 44 years**	**Total Participants**: 200 100 males ,100 females	"Ethnicity or race of participants was not explicitlystated, but given the study location (Port Harcourt, Nigeria)	between infertile and fertile subjects, reproductive hormone imbalance and Vitamin B12 deficiency were pronounced in infertile individuals.

7	**Human male** participants, specifically: Infertile men with oligozoospermia (cases) **Fertile men** with normal sperm parameters (controls)	**Inclusion Criteria:**Age Range: Adults between 18 and 44 years, Health Status: Apparently healthy individuals (no acute illness),Fertility Clinic Attendance**Exclusion Criteria:**outside the reproductive age range (under 18 or over 44) or not attending fertility clinics were implicitlyexcluded.	Age range:**21–45 years**	The study differentiatesresults by **sex (male and female)**	Ethnicity/race was not collected or analyzed as part of the demographic data.	the study evaluated the health status of 200 participants (100 fertile and 100 infertile) based on,Vitamin B12 levels,Folatelevels,Reproductive hormones Haematological parameters
8	**Infertile Algerian men.** Number of participants: 29 men	**Inclusion Criteria:***Infertile men*Infertility has been confirmedafter at least one year of unprotectedregularsexual intercourse withoutpregnancy. **Exclusion Criteria:** An exception was made for cases wheretraditional infertility criteriadid not apply,**1 patient** with impaired**sperm motility** (asthenozoospermia).**Another patient**suffers from **azoospermia**	Age range: **24 to 50 years**	All study participants are **male**	All study participants are of **Amazigh-Arab**ethnicity and from Algeria, specifically from the Batna region, a region located in North Africa	All participants were **infertile men.** 64% of themsuffer from **primary infertility** **Poor sperm**motility (asthenozoospermia). **Azoospermia.**

9	Men suffering from infertility (Infertile men).	**Inclusion Criteria** The study included patients with primary infertility, representing 64% of the participants. All participants had engaged in regularunprotectedsexual intercourse for at least one year and provided writteninformed consent. **Exclusion criteria** were not explicitly mentioned in the availablecontext.	The median age of the study population was 35 years, with a range from 24 to 50 years	Male only, as the study focuses on infertile men	Not explicitlystated, but since all researchers are affiliated with the University of Batna in Algeria, the participants are likely Algerian	All participants were infertile men. No other medical conditions were mentioned
10	Men suffering from infertility with suboptimal semen parameters.	**Inclusion Criteria:** Infertile men Aged between 20 and 40 years Diagnosed with suboptimal semen parameters Followed up at a fertility clinic in a tertiary healthcare center in North India **Exclusion Criteria:** Not explicitly mentioned in the abstract.	Infertile men Aged between 20 and 40 years	Male only > "73 infertile males	Not directly mentioned, but the study was conducted in North India, suggesting the participants were likely Indian.	All participants were infertile Some had vitamin B12 and/or D deficiency Suffered from various suboptimal semen parameters (e.g., sperm count, motility, morphology)

11	The study included 200 infertile men	**Inclusion Criteria:** For infertile men (n = 153): Diagnosed with idiopathic infertility. Had regular, unprotectedsexual intercourse. Provided sufficient screening data and biological samples. For fertile men (n = 184): Recruited from fertility or antenatal clinics. Met the required age criteria (not specified in the excerpt but used as a condition). **Exclusion Criteria:** For infertile men: History of any of the followingconditions: Cancer Cryptorchidism Klinefelter's syndrome Y chromosome microdeletions Vasectomy Obstructive azoospermia Hypogonadotropichypogonadism Mumpsorchitis	Age specified in the study: - Age of infertile men: Between 20 and 45 years. - Age of their partners: Lessthan 40 years.	Male only	Not mentioned	Fertile men:Considered healthy. Infertile men:Diagnosed with idiopathic infertility.

4.3.1. Studied Population Type

Studies **(Chavarro *et al*., 2008; Shankar Bharti & Singh, 2023)** : Included young, healthy women of reproductive age (18–25 years), classified according to AMH hormone levels (high/normal), reflecting a focus on polycystic ovary syndrome (PCOS), a major cause of hormone-related infertility.

Studies **(Banihani, 2017; Rastegar Panah *et al.*, 2024):** Focused on men suffering from infertility, some with varicocele or conditions such as oligozoospermia and idiopathic infertility. Study **(Rastegar Panah *et al*., 2024)** included 832 men, exploring the link between B12 deficiency and male fertility parameters (semen, hormones, weight). Study **(Banihani, 2017)** involved men from various nationalities (India, Japan, Spain, etc.) with different infertility conditions, focusing on the direct relationship between B12 and semen quality.

Population Diversity

- Including both male and female samples, and across different ethnicities and geographical regions, reflects a comprehensive perspective and lends credibility to the results. It supports the hypothesis that B12 deficiency may affect fertility differently depending on biological sex and reproductive status.

4.3.2. Inclusion and Exclusion Criteria

Study **(Shankar Bharti & Singh, 2023)**: Excluded women with chronic diseases or systemic conditions; only generally healthy participants were included.

Study **(Rastegar Panah *et al*., 2024)**: Excluded men who had undergone surgery, received radiation therapy, or taken fertility drugs in the last four years.

Study **(Chavarro *et al*., 2008)**: Excluded women with a history of infertility, those who had undergone sterilization, or who had diseases such as diabetes or were menopausal.

- Ensures reduced interference from other factors that may affect fertility, increasing the reliability of the association between B12 deficiency and infertility.

4.3.3. Age

The general age range was between 18 and 50 years.

Studies **(Chavarro *et al*., 2008; Shankar Bharti & Singh, 2023)**: Focused on women aged 18–25.

Study **(Rastegar Panah *et al*., 2024)**: Included men of a similar age group.

Study **(Rastegar Panah *et al*., 2024)**: Included women aged 24–42.

Study **(Chavarro *et al*., 2008)**: Male participants ranged from 20 to 50 years.

- Focusing on reproductive-age individuals strengthens the ability to assess the actual impact of B12 deficiency on fertility without the confounding effect of natural aging.

4.3.4. Sex

Studies **(Chavarro *et al*., 2008; Shankar Bharti & Singh, 2023)**: Focused on women only, addressing hormonal and ovarian aspects.

Studies **(Banihani, 2017; Rastegar Panah *et al*., 2024)**: Focused on men only, targeting sperm quality and male hormone levels.

- Enables comparison of B12 deficiency effects on fertility in both sexes. In women, the impact is through ovulation and hormonal disorders (e.g., AMH, estrogen), while in men, it is through semen quality and male reproductive hormones (FSH, LH, testosterone).

4.3.5. Ethnicity/Race

Study **(Shankar Bharti & Singh, 2023)**: Participants were Indian women.

Study **(Rastegar Panah *et al*., 2024):** The majority of male participants were Caucasian, with some of Asian, African-Canadian, Middle Eastern, and Hispanic descent.

Study **(Chavarro *et al*., 2008):** Participants were white American women.

Study **(Banihani, 2017)**: Included men from India, Japan, Spain, and other diverse ethnic backgrounds.

Enhances understanding of how B12 deficiency impacts fertility based on dietary, environmental, and genetic differences related to ethnicity. Some ethnic groups may be more prone to B12 deficiency due to vegetarian diets or poor absorption.

4.3.6. Health Status

Study **(Shankar Bharti & Singh, 2023)**: The women appeared healthy, but many showed subtle hormonal imbalances and deficiencies in vitamins D and B12.

Study **(Rastegar Panah *et al*., 2024)**: Reported high rates of overweight and obesity among men (48.2% overweight, 24.1% obese), which themselves impact fertility and can intensify the effects of B12 deficiency.

- Even among individuals considered "healthy," B12 deficiency can lead to silent disruptions that affect fertility.

Obesity increases metabolic stress and complicates the effects of the deficiency.

4.4. Exposure Details: Vitamin B12 Status.

With an emphasis on the definition of deficiency, measurement techniques, assessment timing, and classification criteria, Table 6 (Exposure Details: Vitamin B12 Status) provides comprehensive exposure data on vitamin B12 status from all of the included studies. The methodological differences in the definition and evaluation of B12 status in the context of infertility studies are highlighted in this synthesis **(Table06).**

Table 8. Exposure Details : Vitamin B12 Status.

N°	Definition of vitamin B12 deficiency (cut-off values)	Measurement method (e.g., serum B12, methylmalonic acid, holotranscobalamin)	Timing of assessment (preconception, during fertility evaluation, etc)	Categorization of B12 status (deficient vs. sufficient, or quartiles, etc.)
1	/	Blood samples were drawn for the estimation of D3 and B12. Measurements were taken on the second to fifth day of the cycle.	/	/
2	Vitamin B12 deficiency is defined as serum concentration <148 pmol/L. Optimal levels are between 148 to <701 pmol/L. Elevated levels are defined as ≥701 pmol/L	Serum vitamin B 12 concentrations were assessed from venous blood samples. Analyses conducted at Mount Sinai Hospital and LifeLabs	The study assessed serum vitamin B12 during fertility evaluation. Participants were recruited from a urologic wellness center. The recruitment phase occurred from June 2019 to August 2021.	Serum vitamin B12 is categorized into deficient, optimal, and elevated levels. Deficient: <148 pmol/L; optimal: 148 to <701 pmol/L; elevated: ≥701 pmol/L. Serum B12 concentration was grouped into tertiles for analysis.
3	Vitamin B12 deficiency is characterized by insufficient	/	Check B12 before conception, especially for IVF/IUI. B12	Vitamin B12 deficiency is multifactorial, often due to

	levels of vitamin B12 in the body, which can lead to a range of health problems, including anemia, neurological issues, and impaired reproductive functions.		may improve semen quality and the success of the procedure. Vitamin B12 may improve sperm motility/viability in vitro, suggesting its assessment for assisted reproduction.	malabsorption. Deficiency linked to malnutrition or drug-induced causes.Clinical studies report low B12 levels in various populations. B12 supplementation recommended for maintaining population health. No specific categorization of B12 status mentioned in the contexts.
4 5	/ 5 vitamin B12 deficiency is defined b6y low serum levels, with cut-off va78lues typically set at 200 pg/Ml for deficiency diagnosis. Understanding these values is essential for identifying at-risk individuals and implementing appropriate interventions.	/ Serum vitamin B12 levels were estimated using the chemiluminescence method. Global methylation was determined using the ELISA system.	Dietary information was collected in 1991 and 1995. Follow-up for the study started in 1991 and concluded in 1999. Participants reported pregnancies during the preceding two-year period. Clinical assessment was conducted during fertility evaluation at the institute. Subjects provided personal and family medical history during assessment. Informed consent was obtained before participation in the study.	The study categorized women into five groups based on B vitamin intake levels, including B12, but did not provide specific thresholds for deficiency or sufficiency. Oligozoospermic males have significant vitamin B12 deficiency compared to normozoospermic males. Mean serum vitamin B12 concentration was lower in the case group.No quartile categorization of B12 status was mentioned.
6	Vitamin B12 deficiency can cause neurological issues, anemia, and affect fertility.	Vitamin B12 concentration was measured using an ELISA kit. 50 µl of extracted vitamin B12 standards were used in the assay. The procedure	Vitamin B12 should be assessed during fertility evaluation, as it may	The study did not specify B12 categorization methods. Vitamin B12 levels were compared between infertile

	Levels below 200 pg/mL are considered deficient.	involved adding biotinylated intrinsic factor reagent.	contribute to primary or secondary infertility. Routine monitoring of vitamin B12 is recommended in fertility clinics, as lower levels are often found in infertile individuals, aiding early detection and treatment	and control groups. Significant differences in B12 levels were noted in infertile subjects. No quartile or deficiency classifications were mentioned in the study.
7	The paper highlights the importance of adequate vitamin B12 intake to lower tHcy levels and improve semen quality, especially in men with MTHFR polymorphisms, but recommends referring to medical guidelines for exact deficiency cut-offs.	Serum concentrations of B12 and folate were measured in patients. Blood samples were collected from fasting individuals for analysis.	Samples were collected during 2013-2015 from infertility center patients. Infertile men had not been previously diagnosed with other infertility criteria. Semen samples were collected after three days of sexual abstinence. The assessment involved men with impaired spermatogenesis and control participants.	The paper emphasizes the importance of adequate vitamin B12 intake for health outcomes, especially in men with MTHFR polymorphisms, and suggests future research for clear categorization.
8 9	Vitamin B12 deficiency is defined as levels <200 pg./mL Low vitamin B12 levels were noted in subjects with abnormal sperm parameters. Deficiency may impact sperm function and quality.	/ Serum folate and cobalamin levels were measured using an immuno-	The study assessed infertile males during fertility evaluation. Assessment occurred from May 2020 to July 2021. Subjects were recruited from an infertility. The focus was on suboptimal semen parameters.	Low vitamin B12 levels (<200 pg/mL) indicate deficiency in subjects with low sperm count. 41.6% of subjects with low semen volume had low vitamin B12 levels. A significant association exists between low B12 levels and sperm morphology.

		electrochemiluminescence assay. Blood samples were collected after an 8-hour fast and centrifuge.	The study involved infertile men undergoing fertility evaluation. Semen and blood analyses were routine assessments during infertility investigations. Semen samples were collected after a period of sexual abstinence. The assessment aimed to investigate relationships with semen parameters.	The study found that most infertile men had normal serum vitamin B12 levels, with only 20.69% showing deficiency. While no significant correlation was observed between B12 levels and sperm parameters, dietary intake, particularly of organ meats and Camembert cheese, was positively associated with B12 status.
10	Vitamin B12 deficiency is defined as levels below 200 pg/mL.	Serum vitamin B12 levels were assessed using ELISA. Serum vitamin D levels were assessed using CLIA.	/	Low vitamin B12 is defined as <200 pg/mL. 39.7% of participants exhibited low vitamin B12 levels. The mean serum vitamin B12 level was 243.4 pg/mL. No specific quartile categorization is mentioned in the study.
11	/	Serum folate concentrations were measured in the study. Red cell folate (RCF) levels were also assessed. Vitamin B12 concentrations were included in the measurements. Plasma total homocysteine (tHcy) levels were measured.	Infertile men provided semen samples on different days from blood samples.Fertile men provided semen and blood samples on the same day.	In summary, the study offers insights into B12 levels and male infertility but lacks a clear framework for defining B12 deficiency or sufficiency.

4.4.1 Definition of vitamin B12 deficiency (cut-off values).

The definitions of vitamin B12 deficiency thresholds are inconsistent across studies, complicating direct comparisons between results. Some studies have established clear deficiency thresholds: studies 2, 5, 6, 9, and 10 defined deficiency as levels <200 picograms/ml or <148 picomoles/liter. Studies 1, 3, 4, 7, 8, and 11 do not clearly define deficiency. It has been observed that individuals with abnormal sperm parameters also have low B12 levels, as well as neurological disorders and anemia.

4.4.2 Measurement method (e.g., serum B12, methylmalonic acid, holotranscobalamin).

The method of measuring vitamin B12 represents one of the critical methodological elements in evaluating the relationship between this vitamin and fertility, due to its direct impact on the accuracy of diagnosis and the differentiation between true deficiency and functional deficiency. Upon reviewing the included studies, it becomes clear that there is an almost exclusive reliance on traditional level measurements.

Most of the studies (2, 5, 6, 7, 9, 10) used serum B12 measurement as the primary method.

For example, Study 2 conducted the analysis using serum samples taken from veins, and they were analyzed in accredited laboratories such as Mount Sinai and LifeLabs.

Study 5 used the chemiluminescence method, which is an approved technique but does not accurately detect functional vitamin B12 deficiency.

Studies 6 and 10 relied on the ELISA test, which is common and easy to use but susceptible to factors such as the concentration of transport proteins.

4.4.3 Timing of assessment (preconception, during fertility evaluation, etc).

Most studies have evaluated vitamin B12 status during infertility assessments, allowing for a good clinical context.

Studies 2, 5, 6, 7, 9, and 11 indicate that the assessment was conducted during the fertility evaluation.

Study 1: Between the second and fifth day of the menstrual cycle.

Study 3: Recommends doses before pregnancy, especially before assisted procedures (IVF/IUI).

Some studies (5, 7) included follow-up data or sampling over several years, which enhances the validity of the longitudinal data.

4.4.4 Categorization of B12 status (deficient vs. sufficient, or quartiles, etc.).

There is a lack of standardization in how studies classify vitamin B12 deficiency.

Clear classification:

Study 2: Participants were divided into three groups (deficiency <148 pmol/L; optimal between 148-701; high ≥701), and then into three parts for analysis.

Study 10 and 9: A threshold of <200 picograms/mL was used to determine deficiency, without any further subdivisions.

Ambiguous or absent classification:

Studies 1, 3, 4, 6, 7, 8, 11 did not provide an explicit classification (quarters, thirds, fine thresholds).

Some studies only compared non-reproductive groups and controls without a precise classification of vitamin B12 status.

4.5. Outcome Details: Infertility.

The outcome-related data from the included studies is summarized in Table 7 (Outcome Details: Infertility), which also includes information on the diagnostic criteria or procedures used, the type of infertility evaluated, the fertility outcomes monitored, and the definition of infertility. This summary draws attention to methodological differences in the evaluation of infertility across research examining the influence of vitamin B12 levels **(Table07).**

Table 9. Outcome Details: Infertility.

N°	Definition of infertility used (e.g., inability to conceive after 12 months of unprotected intercourse)	Type of infertility (primary vs. secondary)	Fertility outcomes (e.g., pregnancy rates, time to pregnancy, sperm parameters, ovulation rates)	Diagnostic criteria or methods (e.g., semen analysis, hormonal testing)
1	Although the paper does not formally define infertility, it focuses on how vitamin D and B12 deficiencies may affect fertility in women of reproductive age, especially those with hormonal imbalances or fertility-related conditions.	In summary, while the paper does not explicitly define or categorize the type of infertility, it addresses fertility issues in reproductive-aged women, which could potentially include both primary and secondary infertility cases.	/	Blood samples were taken from reproductive aged women for hormonal testing. Hormones measured included testosterone, FSH, LH, and E2. 25(OH)D levels were analyzed using linear regression analyses. The study compared total 25(OH)D and Vitamin-B12 levels.
2	Infertility is defined in the research paper as the inability to achieve clinical pregnancy after a year of consistent unprotected sexual intercourse between a man and woman assigned to those genders at birth.	In summary, while the paper addresses male infertility and its association with vitamin B 12 levels, it does not differentiate between primary and secondary infertility within the provided contexts. Understanding these distinctions	Male factor infertility accounts for ~30% of infertility cases. Low serum vitamin B12 is linked to testosterone deficiency. Vitamin B12 may improve sperm parameters. Nutritional	Reproductive hormones were analyzed using ELISA assays. Seminal parameters indicate male fecundity and infertility diagnosis. Serum vitamin B12 and reproductive hormones were statistically analyzed.

		is crucial for addressing infertility effectively	assessments could enhance male infertility management.	
3	In summary, while the paper does not provide a specific definition of infertility, it emphasizes the importance of vitamin B 12 in enhancing semen quality, which is a critical factor in male fertility. The general definition of infertility as the inability to conceive after 12 months of unprotected intercourse remains applicable in this context	The provided contexts do not contain information regarding types of infertility (primary vs. secondary).	Vitamin B12 improves sperm count and motility, enhancing fertility outcomes. Positive effects on sperm DNA integrity noted with vitamin B12 treatment. Hyperhomocysteinemia negatively impacts embryo quality in IVF procedures. Infertile men show lower plasma vitamin B12 concentrations compared to fertile men. Limited studies report adverse effects of vitamin B12 on semen quality.	/
4	/	/	438 women reported infertility due to ovulatory disorder during follow-up. Women with ovulatory infertility had longer, irregular menstrual cycles. Multivitamin use inversely associated with ovulatory infertility risk. Regular multivitamin use may decrease ovulatory infertility risk.	The provided contexts do not contain information regarding diagnostic criteria or methods such as semen analysis or hormonal testing.
5	/	The study focuses on male infertility, particularly oligozoospermia, exploring the link between vitamin B12 levels and DNA methylation, while noting that distinguishing primary from secondary	The study does not provide fertility outcomes data. Focuses on vitamin B12 and DNA methylation in infertile males. No significant differences in sperm	Semen analyses were performed according to WHO criteria 2010 . Clinical history included symptoms of hypogonadism and smoking history. Serum vitamin

		infertility may aid in interpreting the findings.	parameters were reported. Hormonal parameters did not differ between groups. Oligozoospermic men showed lower global DNA methylation levels. No correlation found between vitamin B12 and DNA methylation.	B12 levels were estimated using the chemiluminescence method.
6	Infertility is defined as inability to conceive after 12 months of unprotected intercourse. This definition applies to both primary and secondary infertility.	Infertility is classified as primary or secondary based on conception history. Primary infertility occurs when couples have never conceived. Secondary infertility occurs after a previous conception. The study included both primary and secondary infertile participants.	/	Serum prolactin and fertility hormones were analyzed using ELISA technique. FSH and LH concentrations were determined with specific ELISA kits. Vitamin B12 levels were evaluated alongside other reproductive hormones.
7	/	/	Daily intake of vitamins B9 and B12 improved sperm parameters significantly. Higher vitamin B intake correlated with better sperm concentration and motility. The study assessed sperm parameters among men with MTHFR polymorphisms. Sufficient vitamin B consumption influenced fertility in men with T allele.	Semen parameters evaluated an average of three ejaculations. Samples collected after three days of sexual abstinence. Semen samples analyzed for asthenospermia, oligospermia, severe oligospermia, and azoospermia.
8	Infertility is defined as inability to conceive after 12 months. It	/	Reduced vitamin B12 and D levels disturb fertility	Serum vitamin D levels were estimated by chemiluminescence

	involves regular unprotected sexual intercourse during this period.		mechanisms. Vitamin B12 positively affects sperm parameters, especially sperm count. Vitamin D is necessary for optimal male reproductive function. No specific pregnancy rates or time to pregnancy data provided.	method. Serum vitamin B12 levels were also estimated using chemiluminescence method. Semen analysis parameters were compared with vitamin levels using statistical tests. The study involved male infertility cases with suboptimal semen parameters.
9	Infertility is defined as inability to conceive after 12 months of unprotected intercourse. Approximately 15% of couples experience this issue. Up to 50% of infertility cases are due to male disorders.	while the paper does not explicitly categorize the infertility types, it provides valuable insights into factors affecting male fertility that can be relevant to both primary and secondary infertility scenarios	Serum folate levels positively correlated with sperm concentration and motility. Higher folate intake improved sperm parameters in men with specific genetic traits. Consumption of fruits and egg yolk correlated positively with sperm concentration and motility. Infertility affects approximately 15% of couples globally.	Semen quality was analyzed using VideoTesT-Sperm software and optical microscopy. Sperm concentration, motility, and morphology were assessed. Oligozoospermia diagnosed with sperm concentration <15 million/mL. Serum vitamin concentrations measured with immuno-electrochemiluminescence assay.
10	Infertility is defined as inability to conceive after 12 months of unprotected intercourse. This definition is characterized by nonobstructive infertility.	The study does not specify types of infertility. It focuses on male infertility in general. No distinction between primary and secondary infertility is made.	The study found significant differences in sperm count and motility based on vitamin levels. Low vitamin B12 and D levels correlated with suboptimal semen parameters. Addressing nutritional deficiencies may improve male fertility outcomes.	Semen samples were processed to preserve integrity for analysis. Sperm concentration was assessed using a hemocytomet. Sperm motility was evaluated by observing movement patterns. Morphology assessment involved examining sperm size and shape. Viability testing differentiated live and dead

				sperm cells. Semen pH was measured to evaluate acidity or alkalinity. White blood cell counts identified potential reproductive tract inflammation. Standardized WHO techniques ensured accuracy in semen analysis.
11	Infertility defined as inability to conceive after 12 months of unprotected intercourse. Study required regular sexual intercourse without contraception for one year.	/	/	Semen volume, sperm concentration, motility, and morphology were analyzed. Sperm DNA fragmentation was evaluated using the sperm chromatin structure assay. WHO guidelines were followed for semen sample collection and analysis. Blood and semen samples were collected for analysis.

4.5.1 Definition of infertility used (e.g., inability to conceive after 12 months of unprotected intercourse).

The examination of infertility definitions in the studies encompassed by this systematic review indicates considerable variability. Multiple studies, particularly studies 2, 6, 8, 9, 10, and 11, converge on a standardized definition of infertility: the inability to conceive after 12 months of regular, unprotected sexual intercourse. Study 2 applies this definition to male infertility, whereas Study 6 extends it to encompass both primary and secondary infertility. Studies 8 to 11 utilize this framework to organize their clinical analyses, specifically regarding semen parameters. Other studies (1, 3, 4, 5, and 7) do not provide a clear definition of infertility. This inconsistency may impede the direct comparability of findings and highlights the necessity for standardizing diagnostic criteria in future research.

4.5.2 Type of infertility (primary vs. secondary)

The only study that explicitly divides individuals into main and secondary infertility groups is Study 6. On the other hand, primary and secondary infertility are not specifically distinguished in research 1, 2, 3, 4, 5, 7, 8, 9, 10, and 11. Some research looks at infertile males (studies 2, 5, 7, 8, and 9) without mentioning whether or not they had fathered children in the past.

4.5.3 Fertility outcomes (e.g., pregnancy rates, time to pregnancy, sperm parameters, ovulation rates).

Changes in semen parameters are significantly linked to vitamin B12 insufficiency, according to studies 2, 3, 5, 7, 8, 9, 10, and 11:

Sperm DNA integrity: Studies 3 and 11 showed improvements after using vitamin B12 supplements.

Sex hormones: Reduced testosterone levels were linked to lower vitamin B12 levels (study

The impacts on women are less well-researched and more indirect, yet they are nevertheless significant:

According to studies 1 and 4, women who are deficient in vitamin B12 (and maybe vitamin D) may have ovulatory or hormonal problems that could impair fertility. Infertility is also linked to irregular menstrual periods, which are a sign of anovulation, according to Study 4, which also suggests that taking multivitamins, which may include vitamin B12, may lower this risk.

4.5.4 Diagnostic criteria or methods (e.g., semen analysis, hormonal testing).

Numerous standards and diagnostic techniques for determining fertility have been covered in earlier research. While some research (studies 3 and 11) used more sophisticated evaluations, such as DNA fragmentation analysis and sperm chromatin structure assay (SCSA), studies 2, 5, 7, 8, 9, 10, and 11 relied on semen analysis techniques. These tests are especially important since a lack of vitamin B12 may cause DNA damage by means of oxidative stress and increased homocysteine levels (Halczuk et al., 2023). Gonadal hormone levels were also analyzed in trials 2, 5, and 6.

While only a small number of research studies (1, 4, and 6) included female subjects, several used methods like ELISA to measure hormone

4.6. Association/Effect Estimates

Table 8(Association/Effect Estimates) presents a summary of the associations between vitamin B12 status and infertility outcomes across the included studies, highlighting effect sizes, statistical significance, adjustments for confounding variables, and the analytical methods employed. This table provides a comparative overview of how B12-related reproductive outcomes are quantified and interpreted in diverse research settings **(Table08).**

Table 10. Association/Effect Estimates

N°	Main findings related to B12 and infertility	Effect size (e.g., odds ratio, risk ratio, hazard ratio)	Confidence intervals and p-values	Adjustment for confounders (e.g., age, BMI, folate status, socioeconomic factors)	Statistical methods used
1	The study found a negative correlation (r = -0.529) between Vitamin B12 levels and AMH, suggesting that lower B12 levels may be linked to higher AMH in infertile women. It also explored B12 levels in women with high AMH and polycystic ovaries, highlighting a possible connection to infertility	/	/	/	The statistical methods used in the study included linear regression analyses to analyze the associations between 25(OH)D and reproductive hormones, as well as hypogonadism.
2	The study aimed to assess the relationship between serum vitamin	The study found that men in the middle and highest tertiles of serum vitamin B12 had	The odds ratios (ORs) for the association between tertiles of serum vitamin B 12	The study adjusted for several clinically relevant covariates, including age, BMI, current	The study used R Studio for data analysis, applying

	B12 levels and reproductive hormones in infertile men. It found that higher B12 levels were associated with improved androgenic profiles, especially total testosterone, but showed no significant link with sperm parameters. The study also emphasized the need for further research on B12's role in male reproductive hormones.	lower odds of testosterone deficiency compared to those in the lowest tertile. Adjusted odds ratios were 0.48 for the middle tertile and 0.44 for the highest, indicating a protective association.	concentration and reproductive hormones include 95% confidence intervals (CIs) and p-values. For example, the unadjusted OR for elevated LH in the highest tertile is 0.50 (95% CI: 0.28, 0.90, P = 0.02) and in the adjusted model, it is 0.53 (95% CI: 0.25, 1.13, P = 0.05). Additionally, for testosterone deficiency, the adjusted ORs are 0.48 (95% CI: 0.25, 0.93, P = 0.03) for the mid-tertile and 0.44 (95% CI: 0.22, 0.87, P = 0.02) for the highest tertile.	alcohol consumption, current smoking status, meteorological season when blood was drawn, and ethnicity in both multivariate linear and logistic regression models. However, folate status and socioeconomic factors were not mentioned as confounders in the analyses.	chi-square tests and ANOVA to assess group differences, and Spearman's correlation for nonparametric associations. Linear and logistic regression models, both unadjusted and adjusted for covariates like age, BMI, and lifestyle factors, examined the relationship between vitamin B12 levels and reproductive hormones.
3	The review suggests that vitamin B12 may support semen quality and spermatogenesis by enhancing reproductive organ function and reducing oxidative damage. While some studies report improvements in sperm	/	/	/	The provided contexts do not contain any information regarding the statistical methods used in the research.

	count and motility, others found no significant association between B12 levels and semen quality.				
4	The research showed that vitamin B12 intake was inversely related to ovulatory infertility risk but was less significant than folic acid, which was primarily linked to reduced infertility risk.	The multivariate-adjusted relative risk of ovulatory infertility was reported as 0.88 (95% CI: 0.60, 1.28) for women consuming 2 tablets/week or less, 0.69 (95% CI: 0.51, 0.95) for those consuming 3 to 5 tablets/week, and 0.59 (95% CI: 0.46, 0.75) for women consuming 6 or more tablets/week, compared to non-users of multivitamins.	The study reported that higher multivitamin intake was associated with a significantly reduced risk of ovulatory infertility, with relative risks decreasing and a strong trend ($p < 0.001$) supported by 95% confidence intervals.	The study adjusted for multiple confounders—including age, BMI, lifestyle, diet, and iron intake—to account for their potential impact on infertility risk.	The study used logistic regression and generalized estimating equations to assess ovulatory infertility risk related to multivitamin and B-vitamin intake, adjusting for confounders, and calculated population attributable risk to estimate preventable cases.
5	The study found significantly lower vitamin B12 levels in infertile men and women, correlated with decreased fertility hormones, suggesting vitamin B12 should be included in infertility assessments.	/	The research highlighted the need to report effect sizes and confidence intervals with p-values, using a significance threshold of ≤ 0.05, while noting non-significant results like FSH ($p = 0.091$) and the correlation between vitamin	/	The study used Microsoft Excel for data handling and MINITAB 16 for analysis, applying Kolmogorov-Smirnov and Bartlett's tests for

			B12 and methylation defect ($p = 0.76$).		normality and variance, unpaired t-tests with Tukey's post-hoc for group comparisons, and Pearson's correlation to assess vitamin B12 and DNA methylation relationships, with significance at $P \leq 0.05$.
6	The study linked vitamin B12 deficiency to infertility and oligozoospermia, possibly via elevated homocysteine, but found no correlation with global DNA methylation defects.	/	The study found no significant difference in serum folate levels in infertile females ($p = 0.2400$), but vitamin B12 levels were significantly lower in infertile females ($p = 0.0078$) and males ($p < 0.0001$); confidence intervals were not reported.	/	The statistical methods used in the study included the Graph-Pad Prism 8.0.2.263 version statistical package to obtain mean and standard deviation of the study groups. A Student t-test was employed to determine the statistical difference between fertile and infertile

					subjects, with results presented as mean ± standard deviation (M±SD) in tables
7	The study found that daily intake of vitamin B12, along with B9, significantly optimized sperm concentration and motility in men with different MTHFR genotypes, particularly those with the T allele, which is associated with infertility. Additionally, sufficient consumption of these vitamins was linked to improved semen parameters and lower total homocysteine levels, indicating a critical role in male fertility.	/	The study computed the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses to assess the association between different genotypes of polymorphisms and infertility. A p-value of <0.05 was considered statistically significant in the analysis of tHcy concentration differences and linear correlations between B12, folate intake, and serum concentrations.	/	The study used SPSS 19 for analysis, applying logistic regression to assess genotype-infertility associations with odds ratios and 95% CIs, Hardy-Weinberg tests for polymorphisms, Student's t-tests for homocysteine differences by vitamin intake, and Pearson correlation to evaluate relationships between vitamin B12, folate intake, and serum levels.
8	The study concluded that low vitamin B12 levels	/	The study found significant associations between serum	/	Data analysis was conducted using

	in infertile males disrupt fertility-related physiological processes, with significant associations to sperm count ($p = 0.003$) and morphology ($p = 0.049$), possibly impairing sperm function via nitric oxide depletion.		vitamin B12 levels and sperm count ($p = 0.003$) and morphology ($p = 0.049$), but no significant links with semen volume ($p = 0.959$) or sperm motility ($p = 0.069$); confidence intervals were not reported.		SPSS 25.0, applying descriptive statistics for numerical and categorical variables, testing normality with Kolmogorov-Smirnov, and comparing semen and biochemical parameters using Chi-square and Fisher's exact tests, with significance at $p < 0.05$.
9	The study found that consumption of organ meat and Camembert cheese positively correlates with serum vitamin B12 levels, suggesting diet influences B12 status, but it did not directly link B12 levels to infertility outcomes, focusing more on folate's role.	/	The study reported significant differences in sperm concentration ($p = 0.0308$) and progressive motility ($p = 0.0169$) by serum folate levels, and a strong correlation between serum folate and DNA fragmentation index ($p = 0.0068$); confidence intervals were not provided.	/	Spearman correlation coefficients and Mann-Whitney U tests were used to analyze relationships and group differences, with DNA fragmentation index cut-offs at 18 and 30; data were presented as

					median (range) with 95% Cis, analyzed in GraphPad Prism 7, and significance set at $p < 0.05$.
10	The study found a significant link between low serum vitamin B12 (<200 pg/mL) and poorer semen parameters in infertile males, with 39.7% showing deficiency and a mean B12 level of 243.4 ± 69.3 pg/mL, suggesting that correcting B12 deficiency may improve fertility.	/	The study set the significance level at $p = 0.05$ and used Pearson's correlation to examine associations between serum vitamin levels and semen parameters, reporting non-significant p-values for semen volume (0.151) and sperm count (0.740) between low and normal vitamin D groups; confidence intervals were not reported.	/	The study used SPSS 20.0 for analysis, reporting means ± SD and frequencies, with Student's t-tests, Chi-square tests, and Pearson correlations assessing semen parameters and serum vitamin levels at a significance level of $P < 0.05$.
11	The study found lower vitamin B12 levels in infertile men compared to fertile men, but the difference was not statistically significant, and B12 was not a significant predictor of infertility in logistic	The research paper discusses the use of logistic regression to calculate adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between the risk of infertility and metabolite concentrations among non-users of vitamins, as well as for	The study's statistical models adjusted for confounders like age, education, smoking, abstinence length, and parental origin but did not account for BMI or specific socioeconomic factors, focusing mainly on demographic and lifestyle variables.	The study's statistical models adjusted for confounders like age, education, smoking, abstinence length, and parental origin but did not account for BMI or specific socioeconomic factors, focusing mainly on	The study utilized linear regression models to compare semen quality between vitamin users and non-users, adjusting for covariates.

	regression; genetic variants in B12 metabolism genes, like TCblR, showed some association, though overall the research offers limited support for low B12's role in idiopathic male infertility.	case-control comparisons of SNP genotype distributions. However, specific effect sizes (e.g., odds ratios) are not provided in the excerpts reviewed.		demographic and lifestyle variables.	Statistical analyses were performed by specific authors who contributed to the study, ensuring a structured approach to data evaluation.

4.6.1 Main findings related to B12 and infertility

In men, the majority of research has linked low vitamin B12 levels to detrimental alterations in fertility, including

- reduced motility and sperm count (studies 3, 5, 6, 7, 8, and 10).
- An increase in sperm genetic damage (studies 3, 11).
- impact on the levels of male hormones, particularly testosterone (research 2).
- According to some research, vitamin B12 supplementation improved the quality of semen (studies 3, 7).

In females:

Studies 1 and 4 showed a relationship between low B12 levels and ovulatory disorders or high AMH, which are indicators of polycystic ovary syndrome.

One study (4) showed a reduced risk of ovulatory infertility in women who take multivitamin supplements containing B12.

4.6.2 Effect size (e.g., odds ratio, risk ratio, hazard ratio).

The results of some studies within this systematic review indicate the presence of quantitative evidence supporting the effect of vitamin B12 on fertility, particularly through the provision of clear statistical indicators such as odds ratios (OR) and relative risk ratios (RR). For example, Study

2 provided strong evidence of the relationship between B12 levels and testosterone deficiency in men, with an OR for the highest B12 concentration category of approximately 0.44 (95% CI: 0.22–0.87, p=0.02), indicating a clear protective effect against testosterone deficiency. Similarly, Study

7 relied on OR analysis to evaluate the relationship between MTHFR gene mutations and fertility indicators, highlighting the role of vitamin B12 (along with B9) in improving semen parameters in carriers of genetic mutations, especially those carrying the T allele associated with elevated homocysteine and infertility.

In studies that included women, study 4 presented relative risk ratios (RR) indicating a gradual decrease in the risk of ovulatory infertility with increased intake of dietary supplements containing B vitamins, where the risks decreased from RR = 0.88 to 0.59 according to the number of tablets per week, with strong statistical significance.

In contrast, the majority of other studies (3, 5, 6, 8, 9, 10, 11) did not provide direct quantitative estimates such as OR or RR, or were limited to reporting statistical significance (such as p-values) without specifying effect sizes, which restricts the ability to make precise quantitative comparisons between studies and underscores the need for more studies that provide detailed and comparable data suitable for meta-analysis.

4.6.3 Confidence intervals and p-values.

Many studies have provided confidence intervals with statistical significance: Studies 2, 4, and 7 included precise CI and p-values.

Studies 5, 6, 8, and 10 provided only p-values without confidence intervals (CI).

Study 8, for example, mentioned that B12 was significantly associated with sperm count ($p = 0.003$) andmorphology ($p = 0.049$).

Some studies (like 11) did not find statistically significant differences despite the presence of quantitative differences.

4.6.4 Adjustment for confounders (e.g., age, BMI, folate status, socioeconomic factors).

Statistical adjustment for confounders represents a pivotal step in epidemiological studies to determine the true relationship between vitamin B12 deficiency and fertility, and the studies included in this review have shown a clear variation in the strength of this methodological aspect.

In Study 2, a precise and comprehensive adjustment was applied to the statistical models, taking into account influencing factors such as age, body mass index (BMI), alcohol consumption, smoking, and the season in which the analysis was conducted, which enhances the reliability of the results and reduces the likelihood of bias.

Similarly, Study 4 paid sufficient attention to this aspect, as it adjusted for a range of lifestyle-related factors, dietary intake, and iron and vitamin consumption, which is a notable strength when analyzing the relationship between dietary intake and the risk of ovulatory infertility.

In contrast, some other studies have shown a systematic deficiency in addressing confounding factors. For example, studies 5, 6, 8, 9, and 10 did not adequately clarify whether they adjusted for these variables, which could lead to exaggerating or underestimating the actual correlation between vitamin B12 levels and fertility indicators. Additionally, study 11, despite using statistical regression models, did not include body mass index or economic factors among the adjusted factors, limiting itself to demographic variables such as age, smoking, and duration of abstinence from intercourse, which weakens the ability to interpret the results within a clear causal context.

Therefore, the variation in this aspect is one of the most important methodological strengths and weaknesses among the studies, and it emphasizes the necessity of including key confounding factors in future designs to obtain more accurate and reliable estimates of the relationship between vitamin B12 deficiency and infertility.

4.6.5 Statistical methods used

For Study 2: Logistic analysis, linear regression, and non-parametric tests were used. Study 7: Used SPSS, correlation and regression tests, and gene tests.

Study 4: Used Generalized Estimating Equations (GEE) and logistic regression. Limited methods in others: Some studies used simple analysis tools like Excel (Study 5) or only GraphPad (Studies 6, 9). Study 3 did not clarify the tools or methods used.The existence of variation in the type of statistical analysis reflects variation in the strength of the results and their generalizability.

4.7. Additional Relevant Findings

This table presents the additional analytical aspects addressed in the 11 included studies. It covers subgroup analyses, interactions between nutrients, and mechanistic (physiological) insights discussed within the studies **(Table09).**

Table 11. Additional Relevant Findings

N°	Subgroup analyses (e.g., sex-specific, age groups, geographic differences)	Interaction with other nutrients (e.g., folate, iron, homocysteine levels)	Mechanistic insights (if any are discussed, such as B12's role in DNA synthesis, ovulation, spermatogenesis)
1	The study focused on reproductive-aged women (18–25 years), comparing two groups based on AMH levels: Group-A (40 women with high AMH) and Group-B (60 women).	/	/
2	The study primarily examined age as a covariate, noting significant differences across vitamin B12 tertiles (P = 0.03), but found no age–vitamin B12 interaction and did not explore sex or geographic subgroup differences.	The study highlights the link between hyperhomocysteinemia, often due to low vitamin B12/folate—and impaired male fertility, emphasizing methylcobalamin's role in homocysteine metabolism.	Vitamin B12 is essential for DNA synthesis, red blood cell maturation, and spermatogenesis, potentially enhancing sperm health and fertility by supporting homocysteine remethylation and reducing inflammation-related sperm damage.
3	/	The study highlights a key interaction between vitamin B12 and homocysteine, showing that B12 deficiency reduces methionine synthase activity, leading to hyperhomocysteinemia and associated reproductive issues, particularly affecting sperm health, with no noted	Vitamin B12 is vital for DNA synthesis and the SAM cycle via its role as a coenzyme in homocysteine metabolism, with its deficiency linked to reproductive disorders and impaired semen quality through elevated homocysteine levels and reduced reproductive organ function.

		interactions with other nutrients like folate or iron.	
4	The study focused on the relationship between multivitamin use and ovulatory infertility, finding no significant interactions across age, parity, alcohol use, iron supplementation, or BMI, and did not include sex- or geography-based subgroup analyses.	The study found that folic acid intake was linked to a reduced risk of ovulatory infertility, while adjusting for iron intake weakened the association with multivitamin use; overall, folate and iron showed notable interactions affecting infertility risk.	The research discusses the role of folic acid, a B-vitamin, in the context of ovulatory infertility, suggesting it may mediate the association between multivitamin use and ovulatory function. However, specific mechanistic insights regarding B12's role in DNA synthesis, ovulation, or spermatogenesis are not provided in the paper. Additionally, it is noted that carriers of a specific MTHFR gene variant may have decreased ovarian responsiveness, which could relate to folate metabolism, but again, no direct mention of B12 is made.
5	/	Vitamin B12 deficiency is associated with elevated homocysteine levels, which can lead to reproductive disorders and oligozoospermia, indicating a significant interaction between vitamin B12 and homocysteine levels. High homocysteine concentrations have been shown to inversely affect fertility outcomes, suggesting that the interaction between vitamin B12 and homocysteine is critical for reproductive health. The study highlights the importance of vitamin B12 to homocysteine levels, but does not specifically address interactions with other nutrients like folate or iron.	Vitamin B12 is vital for one-carbon metabolism and DNA methylation, with lower levels observed in oligozoospermic males, suggesting a link to male infertility; however, no significant association was found with global sperm DNA methylation or key hormonal parameters.

6	The study included a demographic analysis of participants categorized by sex, with equal representation of 100 males and 100 females, aged 18 to 44 years, highlighting both fertile and infertile groups within each sex. The findings indicated no statistically significant differences in haematological parameters between the male and female test and control groups.	The research indicates that vitamin B12 deficiency, alongside serum folate deficiency, can lead to infertility by causing changes in ovulation and implantation processes. Additionally, low maternal folate status during pregnancy is linked to adverse health outcomes for infants, suggesting an interaction between folate and vitamin B12 in reproductive health. The study emphasizes the importance of evaluating vitamin B12 and folate levels in infertility assessments.	The research paper discusses that prolonged vitamin B12 deficiency can lead to infertility by causing changes in ovulation, development of the ovum, and changes leading to defective implantation. Additionally, vitamin B12 is noted to play a role in the metabolism of every cell in the body, which is essential for energy production and fatty acid synthesis.
7	/	The research indicates that MTHFR variants interact with micronutrients such as folate and B12, influencing metabolic pathways and sperm parameters in men with different MTHFR polymorphisms. Additionally, the study found a significant effect of B12 and B9 vitamin intake on total homocysteine levels and sperm parameters among men with MTHFR polymorphisms. The investigation highlights the critical role of sufficient consumption of vitamins B9 and B12 in improving sperm parameters and reducing total homocysteine levels.	The research discusses the critical effect of vitamin B12 intake on improving semen parameters among men with MTHFR polymorphisms, particularly those with the T allele of the C677T polymorphism. It highlights that sufficient consumption of vitamins B9 and B12 influences sperm parameters, suggesting a role in spermatogenesis.
8	The study included male subjects aged 25-40 years with a history of infertility after unprotected intercourse for more than one year.It focused on infertile males with suboptimal semen parameters, indicating a specific age	The research indicates a possible interaction between vitamin B12 and homocysteine levels, suggesting that low vitamin B12 can lead to increased homocysteine toxicity, which may negatively affect sperm function due to reduced methionine synthesis from homocysteine.	The study discusses the role of vitamin B12 in spermatogenesis, suggesting that it may be transferred from the blood to male reproductive organs, indicating its potential importance in sperm production. Additionally, it is noted that vitamin B12 deficiency could impair sperm function

	group and sex (males). The research was conducted in a tertiary care hospital located in the eastern zone of India, highlighting a geographic focus. There are no specific subgroup analyses mentioned regarding other age groups or broader geographic differences beyond the eastern part of India.	Additionally, vitamin B12 deficiency may reduce sperm function through mechanisms involving nitric oxide depletion, which is also influenced by homocysteine levels.	through mechanisms involving hyperhomocysteinaemia and nitric oxide depletion, which are crucial for sperm motility. The paper also highlights that low vitamin B12 levels can reduce the catalytic activity of methionine synthase, affecting sperm health.
9	/	The study discusses the positive correlation between serum folate concentrations and sperm parameters, indicating an interaction between folate and semen quality. Additionally, it mentions that higher intake of folate and vitamin B12 significantly improved sperm concentration and motility, suggesting a potential interaction between these nutrients in influencing reproductive health. Furthermore, the paper highlights that the dose and duration of folate supplementation can affect sperm DNA fragmentation, indicating a complex interaction between folate and sperm quality.	The research paper discusses the complexity of cobalamin (vitamin B12) metabolism, indicating that its role in biological processes, including spermatogenesis, may be influenced by genetic variability among individuals. This suggests that B12's involvement in sperm health could be more intricate than previously understood. Additionally, the paper highlights the importance of folate to DNA synthesis and its positive correlation with sperm parameters, which may indirectly relate to B12's role in these processes.
10	The study included a specific age group of infertile males, aged 20-40 years, with a mean age of 32 years. There is no mention of sex-specific analyses or geographic differences in the provided contexts. The focus of the study was primarily on the association between serum vitamin B12 and D levels and semen	/	The study highlights a significant positive correlation between vitamin B12 levels and sperm concentration, suggesting its potential role in spermatogenesis and optimal sperm production. Additionally, vitamin B12 was associated with improved sperm morphology, indicating its involvement in maintaining sperm structural integrity. However, the paper does not specifically discuss B12's role in DNA synthesis or ovulation.

	parameters in the specified cohort of infertile males.		
11	/	The study suggests that abnormal folate metabolism, particularly the MTHFR 677C>T variant, may contribute to idiopathic male infertility, but found no significant links between vitamin B12 or homocysteine levels and semen parameters, highlighting mixed evidence and limited research on nutrient blood levels in infertile men.	The research paper does not provide specific mechanistic insights into the role of vitamin B12 in DNA synthesis, ovulation, or spermatogenesis. It mentions that B12 is an essential component of one-carbon metabolism, being a cofactor in the folate-dependent conversion of homocysteine to methionine, but does not elaborate on its mechanistic roles in the contexts requested. The study primarily focuses on the association between folate, vitamin B12, and idiopathic male infertility rather than detailing the mechanisms involved.

These studies highlight the existence of a complex relationship between vitamin B12 deficiency and fertility, through the analysis and interpretation of the published scientific literature across three main axes: subgroup analyses, interactions with other nutrients, and potential biological mechanisms.

4.7.1. Subgroup Analyses

Most studies did not conduct comprehensive subgroup analyses despite the diversity of the studies, which reduces the ability to generalize the results to various age groups and geographical backgrounds. Some studies, such as Study No. 1, were limited to narrow age groups (women aged 18–25 years), analyzing AMH levels (Anti-Müllerian Hormone), a fertility marker, but without including a comparison between the sexes.

Study No. 6 analyzed and compared (100 males and 100 females), showing no significant blood-related differences, but it did not explain hormonal or tissue-level differences.

Study No. 8 was limited to men from Eastern India, which provides an important geographical dimension, but it is not generalizable.

- There is a clear lack of precise subgroup analyses that take into account gender, age, genetic background, nutritional background, and geographical location, even though these may significantly affect the relationship between B12 and fertility. It is important to expand future analyses to identify individual differences and the influence of demographic factors.

4.7.2. Interactions with Other Nutrients

Most studies indicated a significant interaction between vitamin B12 and other nutrients, especially with homocysteine as a key factor in fertility.

Studies No. 2, 3, 5, and 8 discussed that B12 deficiency leads to homocysteine accumulation, as the enzyme methionine synthase, which is responsible for converting homocysteine into methionine, is disrupted — negatively affecting male fertility (sperm strength, count, and motility).

Studies No. 4, 6, and 9 pointed to the interaction of B12 with folic acid, which is logical because they function in the same methylation pathway. A deficiency in one may exacerbate the effects of the other other studies (such as 4 and 7) noted that this interaction worsens in cases of combined folate deficiency or when genetic variants (such as MTHFR C677T) impair the metabolism of B12 and folate, which may explain the variation in fertility outcomes among individuals.

- These results highlight the close interaction between vitamin B12, folate, and homocysteine, and this relationship directly affects reproductive health, particularly in cases of unexplained infertility. Poor integration between these nutrients contributes to reduced semen quality and ovulation disorders. The recommendation is to focus on jointly assessing the levels of these nutrients when evaluating infertility cases.

4.7.3. Mechanistic Insights

Most studies concluded that vitamin B12 plays a crucial role in several biological pathways related to the reproductive system, including DNA synthesis and proper cell division, which are essential for the development of oocytes and sperm.

In studies No. 2, 3, 5, 8, and 10, it was observed that B12 deficiency weakens the action of the methionine synthase enzyme, leading to elevated homocysteine levels and affecting genetic methylation (DNA methylation), and consequently, the development of germ cells.

Study No. 8 added a new mechanism: B12 deficiency leads to a decrease in nitric oxide (NO), which negatively affects sperm motility and blood flow in reproductive organs.

Some studies (No. 6 and 9) indicated the role of B12 in mitochondrial function and the Krebs cycle, through its impact on the energy needed for sperm motility and early embryonic development.

Although Study No. 11 did not find a direct link between B12 and semen quality, it discussed its role in the one-carbon metabolism pathway, which indirectly affects fertility.

Conclusion

Conlucsion

In general, this research shed light on exploring the complex relationship between vitamin B12 deficiency and human fertility, both in men and women, through a systematic review and critical analysis of a set of clinical and epidemiological studies. The results we reached showed that vitamin B12 is not merely a secondary dietary element, but rather plays a fundamental role in a range of biological functions directly linked to human reproductive capacity. It was found that this deficiency may lead to disturbances in sperm quality, ovulation disorders, as well as an increased risk of miscarriage and fetal abnormalities. The results also indicate that vitamin B12 supplementation may improve certain aspects of fertility, including semen quality and menstrual cycle regularity.

The extracted data suggest that this vitamin deficiency can lead to various fertility disorders, such as poor sperm quality in males (low count, poor motility, and abnormal morphology), irregular menstrual cycles, and delayed or absent ovulation in females. This deficiency has also been linked to increased homocysteine levels, a substance that may negatively affect embryo implantation and increase the risk of miscarriage. Some studies highlight that correcting this deficiency through supplementation may improve fertility indicators, supporting the idea of including vitamin B12 as a complementary element in infertility treatment protocols.

Nevertheless, these results should be approached with caution, due to variations in study designs and differences in the evaluation criteria used, in addition to the limited number of randomized controlled clinical trials available. This calls for the reinforcement of scientific research in this field through more consistent and comprehensive future studies that take into account individual differences (such as gender, age, dietary patterns, and health environment) and use accurate biological markers to assess vitamin B12 status and associated reproductive functions.

This study also recommends including vitamin B12 level testing as part of routine checkups for couples suffering from delayed conception, especially in cases where no clear organic causes for infertility are present. Healthcare providers should also work on raising nutritional awareness regarding the importance of this vitamin, particularly among groups at risk of its deficiency, such as vegetarians, pregnant women, and individuals with intestinal or chronic diseases affecting its absorption.

In conclusion, this work confirms the importance of the interdisciplinary approach between nutrition and reproductive health and draws attention to vitamin B12 as a critical biological factor that deserves greater attention from researchers, physicians, and health policy planners in order to support fertility and prevent reproductive disorders more effectively and comprehensively.

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الملخص:

مقدمة: يعتبر العقم من الامراض، ويعرف على انه عدم تحقيق الحمل بعد فترة من الجماع دون استخدام وسائل منع الحمل، حيث يمكن ان تتأثر خصوبة الانسان بالنظام الغذائي ونقص الفيتامين ب 12 يلعب دورا مهما في. **الهدف:** الهدف الرئيسي تقييم ما إذا كان لنقص الفيتامين ب 12 له علاقة مع العقم لدى الانسان. **الطرق:** تم اجراء مراجعة منهجية للأدبيات باتباع إرشادات PRISMA**باستخدام** معايير شاملة لاختيار تجربة عشوائية ودراسات جماعية مستقبلية. تمت مراجعة قواعد البيانات Wiley, PubMed, ScienceDirect لتحديد الدراسات ذات الصلة. **نتائج:** تظهر الدراسات ان لنقص الفيتامين ب 12 تأثير الخصوبة وكذا على حركة وعدد وتشكل الحيوانات المنوية وأيضا على الهرمونات الذكرية وبشكل خاص التستسترون، وأيضا يؤدي الى اظطرابات التبويض والحمل.

الكلمات المفتاحية: فيتامين ب 12، العقم عند الذكور، العقم عند الاناث، تحليل السائل المنوي، تكسر الحمض النووي.

Abstract:

Introduction: Infertility is considered a medical condition and is defined as the inability to achieve pregnancy after a period of regular, unprotected sexual intercourse. Human fertility can be influenced by diet, and vitamin B12 deficiency plays an important role in this context. **Objective:** The main objective is to evaluate whether vitamin B12 deficiency is associated with infertility in humans**. Methods:** A systematic review of the literature was conducted following PRISMA guidelines, using comprehensive criteria to include randomized trials and prospective cohort studies. The databases Wiley, PubMed, and ScienceDirect were searched to identify relevant studies. **Results:** Studies show that vitamin B12 deficiency affects fertility, particularly sperm motility, count, and morphology, as well as male sex hormones, especially testosterone. It also contributes to ovulation and pregnancy disorders.

Keywords:

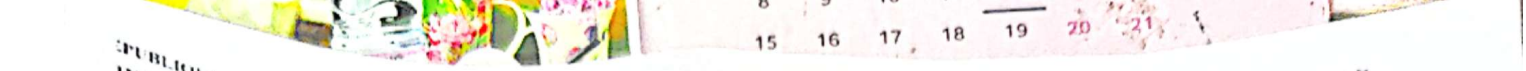
Vitamin B12, male infertility, female infertility, semen analysis, and DNA fragmentation.

Résume :

Introduction : L’infertilité est définie comme l’absence de grossesse après des rapports sexuels réguliers sans contraception. La fertilité humaine peut être influencée par l’alimentation, et la carence en vitamine B12 joue un rôle important. **Objectif :** Évaluer si une carence en vitamine B12 est liée à l’infertilité chez l’être humain. **Méthodes** : Une revue systématique de la littérature a été réalisée selon les directives PRISMA, incluant des essais randomisés et des études de cohorte prospectives. Les bases Wiley, PubMed et Science Direct ont été consultées. **Résultats :** La carence en vitamine B12 affecte la fertilité en réduisant la mobilité, le nombre et la morphologie des spermatozoïdes, ainsi que les hormones sexuelles masculines, notamment la testostérone. Elle peut aussi causer des troubles de l’ovulation et de la grossesse.

Mots-clés :

Vitamine B12, infertilité masculine, infertilité féminine, analyse du spermee fragmentation de l'ADN.



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* المذكرة تتوافق بشكلها الحالي مع النموذج المعتمد لقسم علوم الطبيعة والحياة.
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