

THE IMPACT OF ANTI-MÜLLERIAN HORMONE ON INFERTILITY IN MARRIED COUPLES

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Abstract

Introduction: Anti-Müllerian hormone (AMH) is produced by the granulosa cells of small follicles growing in the ovaries. Serum AMH levels correlate strongly with the number of growing follicles, and therefore AMH has been increasingly recognized as a valuable hormonal marker for the ovaries. Anti-Müllerian hormone (AMH) is a member of the transforming growth factor beta family that takes its name from its role in male sex differentiation by promoting regression of the Müllerian ducts.

AMH is best known as a serum marker of ovarian function and as an estimator of AMH values as a hormone for ovarian reserve for the detection of polycystic ovary syndrome.

Aim of the study: The main purpose of this paper is the prevalence of infertility in married couples for primary and secondary infertility.

This paper aims to investigate the prevalence of infertility in married couples in women for Anti-Müllerian hormone and in men for sperm quality, sperm count and their pathologies in a spermogram by expressing them in percentage and the prevalence of infertility by taking anamnesis from both women and men in married couples of different age groups.

Material and method: In this study, sperm samples from men of infertile couples were analyzed, determining the amount of sperm the number of spermatozoa and their motility in men with primary and secondary infertility. In women, in addition to the general anamnesis, an analysis of the Anti-müllerian hormone was performed by taking the blood (serum) of the patients in the study and the measurements of this hormone were tested with the (ELFA) fluorescent immunoassay method with the aparature Vidas.

Results: The results of the research will consist of the prevalence of primary and secondary infertility and the level of Anti-müllerian hormone in married couples.

The results of the research will also provide a real picture of the situation of married couples suffering from this syndrome. From the research results, recommendations will be given on improving infertility in married couples as a phenomenon that has recently become very concerning for married couples affected by primary and secondary infertility.

Conclusion: Male infertility is expressed by a high incidence of decreased sperm quality and sperm damage manifested by asthenozoospermia and teratozoospermia. The level of Anti-müllerian hormone is lower in women in the study with secondary infertility compared to patients in the control group and patients with primary infertility.

This paper will serve the field of Gynecology in particular and the field of medicine in general.

Keywords: infertility, semen analysis, primary and secondary infertility, Anti-Müllerian hormone.

1. Introduction

Infertility is a global health issue affecting approximately 8-10% of couples. It is a multidimensional problem with social, economic and cultural implications (RoupaZ. et al., 2009).

Infertility is defined as the inability of couples to conceive, both may have disabilities (American Society for Reproductive medicine, 2021 ASRM). The man was considered infertile after one year of normal sexual intercourse if this happened for the first time after marriage it was considered primary infertility, but if it happened after a previous pregnancy it is called secondary infertility. Infertility in women is more likely than men to take personal responsibility for the difficulties of conception (American Society for Reproductive medicine, 2021 ASRM). The worldwide infertility rate is 8–12% (Tasci E, et al., 2008).

The spermiogram is the first-line test for male infertility it assesses the macroscopic and microscopic characteristics of the sperm (WHO, 2010).

The evaluation of infertility in males consists of physical examination and semen analyses. Standardized semen analyses depend on the descriptive analysis of sperm motility, morphology, and concentration, with a threshold level that must be surpassed to be considered a fertile spermatozoon. Nonetheless, these conventional parameters are not satisfactory for clinicians since 25% of infertility cases worldwide remain unexplained (Khatun A et al., 2018).

Conventional semen analysis is commonly used to define semen quality and to predict only quantitative values (Esteves SC, 2010).

Conventional semen analysis is considered as the initial step to investigate semen quality and male factor infertility; however, this method cannot always provide valid information regarding specific defects of sperm physiology. Therefore, novel predictors are needed for assessing semen quality to determine the reason for non-pregnancy in infertile couples (Khatun A et al., 2018).

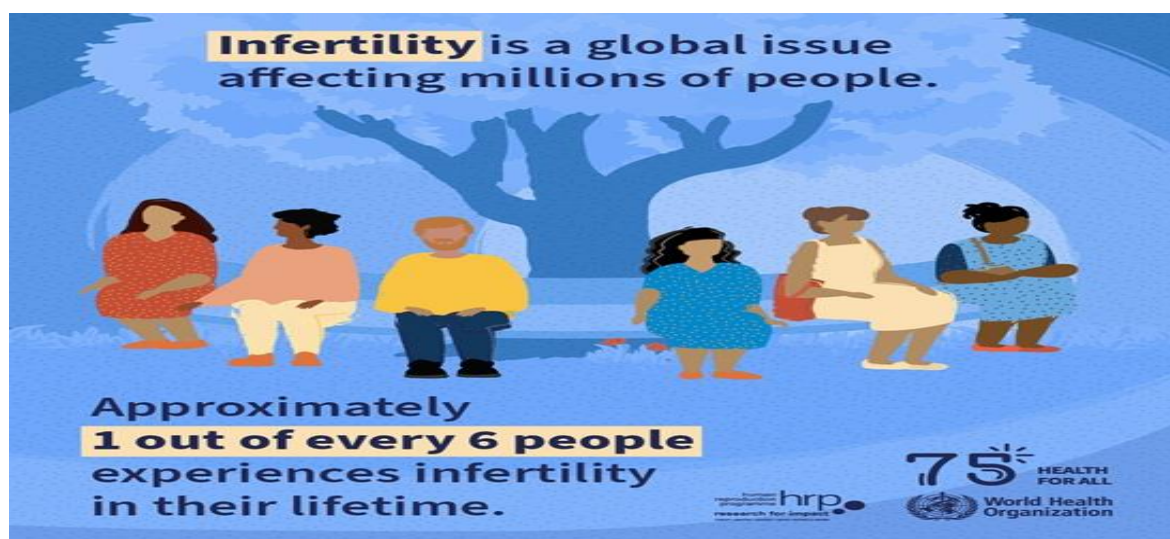


Figure 1: Infertility according to WHO (WHO, 2024)

Anti-Müllerian hormone (AMH) is produced by the granulosa cells of small growing follicles in the ovary. Serum AMH levels are strongly correlated with the number of growing follicles, and therefore AMH has received increasing attention as a marker of ovarian reserve (Moolhuijsen LME, Visser JA., 2020).

After follicle-stimulating hormone (FSH)-dependent selection, AMH expression disappears, although some expression remains in the cumulus cells of preovulatory follicles. Also, in atretic follicles and corpora lutea, AMH expression is lost (Jeppesen JV et al., 2013).

Anti-Müllerian hormone appears to be the best endocrine marker for assessing age-related ovarian reserve decline in healthy women thus, it has potential to predict reproductive longevity. However, it should be noted that only sparse longitudinal data are available in this area, and the

clinical use of AMH in counseling individual women outside the context of IVF is not well supported (Gurgan Clinic, 2025).

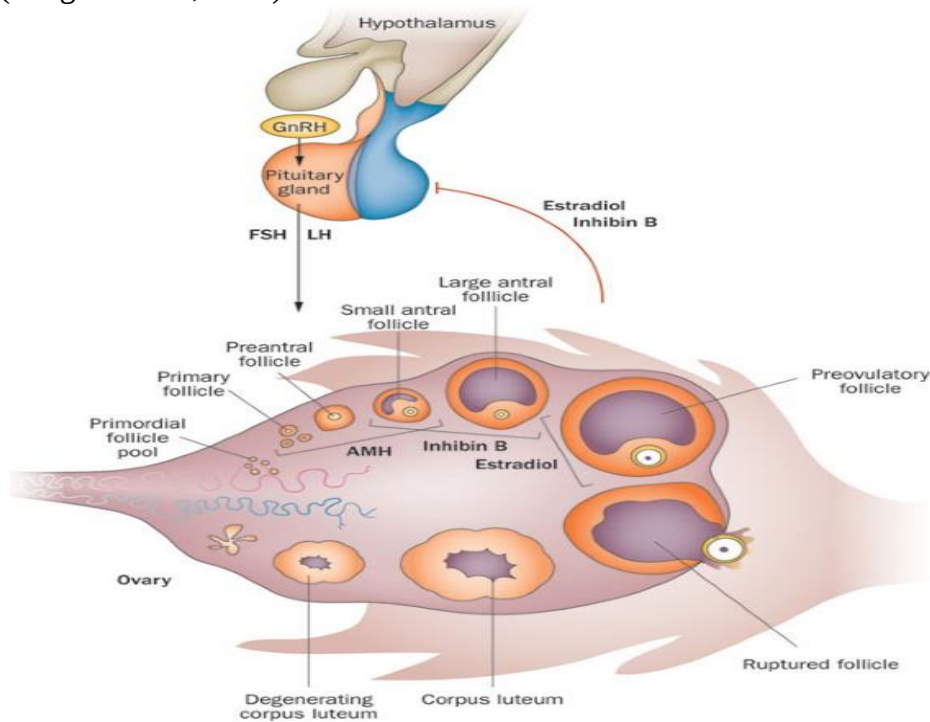


Figure 2: Regulation of Anti-Müllerian hormone associated with ovarian reserve (Jenny A.Visser, 2012)

Anti-Müllerian hormone (AMH) is an important indicator of ovarian reserve and is used to assess fertility potential in women. Low levels of AMH may indicate decreased ovarian reserve, which may reduce fertility potential. Conversely, high levels of AMH are often associated with conditions such as polycystic ovary syndrome (PCOS), where there is an increased number of small, underdeveloped follicles, resulting in excessive production of AMH. This association between AMH levels and various ovarian conditions highlights the importance of measuring this hormone to assess and manage fertility problems (Gurgan Clinic, 2025).

Obesity, primarily characterized by excessive fat accumulation, is a multifactorial chronic disease with an increasing global prevalence. Despite the well-documented epidemiology and significant advances in understanding its pathophysiology and clinical implications, the impact of sex is typically overlooked in obesity research. Worldwide, women have a higher likelihood to become obese compared to men. Although women are offered weight loss interventions more often and at earlier stages than men, they are more vulnerable to psychopathology. Men, on the other hand, are less likely to pursue weight loss intervention and are more susceptible to the metabolic implications of obesity (Koceva A et al., 2024).

In observational studies of men, decreased Vitamin D levels have been associated with poor semen quality, low sperm count and abnormal motility and morphology (Lerchbaum E et al., 2012), (Blomberg Jensen M et al., 2011). The function of vitamin D in male fertility is not known, but may be related to the regulation of calcium (Uhland AM et al., 1992). In women, observational fertility-related research has focused primarily on endometriosis and polycystic ovarian syndrome (PCOS). Data from observational studies suggest that the vitamin D regulatory network is involved in the pathogenesis of endometriosis through the dysregulation and over expression of Vitamin D Receptor in the endometrium, and Vitamin D deficiency is associated with insulin resistance and PCOS (Yildizhan R et al., 2009), (Wehr E et al., 2009), (Agic A et al., 2007), (Somigliana E et al., 2007).

2. Material and method

In this study, sperm samples from men of infertile couples were taken and further analyzed by determining the amount of sperm the number of spermatozoa and their motility in men with primary infertility and secondary infertility by microscopy.

In women, in addition to the general anamnesis, an analysis of the Anti-müllerian hormone was performed by taking the blood (serum) of the patients in the study. The blood samples were centrifuged using the Hettich Universal 1200 centrifuge and the measurements of this hormone were worked out with the (ELFA) fluorescent immunoassay method Vidas (biomerieux).

The first group will include patients aged: 25-49 years the second group patients aged >50 years.



Figure 3: **Hettich Universal 1200 centrifuge and Mini Vidas Biomerieux** (Hettich Universal centrifuge and Mini Vidas-Albimedica laboratory, 2025)

Blood collection guide protocol

Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. The equipment required includes:

- a supply of laboratory sample tubes, which should be stored dry and upright in a rack; blood can be collected in sterile glass or plastic tubes with rubber caps (the choice of tube will depend on what is agreed with the laboratory); vacuum-extraction blood tubes; or glass tubes with screw caps
- a sterile glass or bleeding pack (collapsible) if large quantities of blood are to be collected
- well-fitting, non-sterile gloves
- an assortment of blood-sampling devices (safety-engineered devices or needles and syringes, see below), of different sizes
- a tourniquet
- alcohol hand rub
- laboratory specimen labels
- writing equipment
- laboratory forms
- leak-proof transportation bags and containers
- a puncture-resistant sharps container

First put on well-fitting, non-sterile gloves. Unless drawing blood cultures, or prepping for a blood collection, clean the site with a 70% alcohol swab for 30 seconds and allow to dry completely (30 seconds). Extend the patient's arm and inspect the antecubital fossa or forearm.

Locate a vein of a good size that is visible, straight and clear. The median cubital vein lies between muscles and is usually the most easy to puncture. Under the basilic vein runs an artery and a nerve, so puncturing here runs the risk of damaging the nerve or artery and is usually more painful. Do not insert the needle where veins are diverting, because this increases the chance of a haematoma. The vein should be visible without applying the tourniquet. Locating the vein will help in determining the correct size of needle.

Apply the tourniquet about 4–5 finger widths above the venepuncture site and re-examine the vein. Anchor the vein by holding the patient's arm and placing a thumb below the venepuncture site. Ask the patient to form a fist so the veins are more prominent.

Enter the vein swiftly at a 30 degree angle or less, and continue to introduce the needle along the vein at the easiest angle of entry.

Withdraw the needle gently and apply gentle pressure to the site with a clean gauze or dry cotton-wool ball. Ask the patient to hold the gauze or cotton wool in place, with the arm extended and raised (WHO, 2010).

Sperm sample for a lab test guide protocol

Refrain from ejaculating for 2-3 days before collecting your sample. This will ensure that your sperm count and sperm mobility are at optimal levels at the time of the sample. For the same reason, do not refrain from ejaculating for more than 5 days.

This doesn't mean you have to avoid sexual activity completely. This only means you have to refrain from ejaculating in this timeframe.

Inform the doctor of any medications you're currently taking. Some prescription drugs and herbal medicines may impact sperm count or other sperm analysis values. Your doctor or lab technician will be able to tell you if you need to discontinue these medications in order to ensure an accurate analysis.

You may also be asked to refrain from drinking alcohol or taking recreational drugs in the days leading up to your sperm sample.

Your sample must be deposited in a sterilized, sealed container in order to get accurate results. Do not use an alternative container, like Tupperware or a sealable plastic bag, to store your sample. The lab will probably provide you with a means of transporting the container with the sample as well. If they don't, be sure to ask what precautions you're required to take when returning your sample to the lab. Make an appointment to return your sample within 1 hour. If the sample is more than an hour old, sperm mobility will have decreased so significantly that it can't be reliably analyzed. Make sure to plan the production and delivery of your sample around a specific appointment time for best results.

Don't forget to take things like traffic and the time it will take to reach the lab into consideration when planning your sample production.

The lab may provide sterilized cleaning wipes or towelettes that you can safely use without affecting the sample. Obtain the sample with masturbation without using lubricants or gels. Lubricants, gels, or even saliva may contaminate your sample and make it unreliable to analyze. Do not collect your sample in a condom or use spermicide as a masturbatory aid. If you need to provide your sample via intercourse, the lab may be able to provide a nontoxic condom that you can use and that won't kill the sperm in the sample. After ejaculating, seal the condom with the provided twist tie and place it in the container. Avoid interfering with the contents of the container afterwards. Do not place your fingers or any foreign objects inside the container, as this will interfere with the analysis results. Leave any pubic hair or clothing thread that falls into the container inside and inform a lab technician about it when you turn in your sample. The lab will be able to remove the hair or thread with a sanitized tool that won't affect the test results. Place the lid on the container and put it in the plastic bag provided. For best

results, seal the container as soon as possible to limit the possibility of contamination. If the lab did not provide you with a bag for storing the container, place it upright in a plastic bag and keep it close to your body. Write your name, date of birth, and time of sample on the container. Write this information clearly with a pen or marker that won't run if the ink gets wet. Make sure this information is accurate, especially the time of sample, as incorrect information may lead to unreliable test results. You may also be asked to provide additional information, such as the number of days of abstinence that preceded the sample. Bring the container back within 1 hour, keeping it close to your body. The first portion of the ejaculate is often the richest in sperm numbers, so it's important to let the lab technician know if your sample is missing this portion. If any of the semen is spilled, do not try to put it back in the container yourself. This will only lead to your inadvertently contaminating the sample.

Spermogram test

	Results	Reference Values (WHO 2021)
Ejaculation volume (ml)	1.4 ml	1.4 (1.3-1.5) ml
pH value	7.1	≥ 7.2
Liquidation	15 minutes	< 1h
Total sperm count	20 milion	30–928 milion
Mobility %	45 %	55% with fast mobility
Progressive mobility %	35 %	32–75 %
Immobile %	20 %	2–5 %
WBC	20-25	1-2

OLIGOZOOSPERMIA- 20 milion spermatozoids

Coment: 10% spermatozoids with Teratozoospermia, 18% spermatozoids with Astenozoospermia

Male factors which include: Reduced sperm count, sperm motility, dead sperm, viscosity, pus cells (WBC) – Inflammation, poor intercourse, distortions.



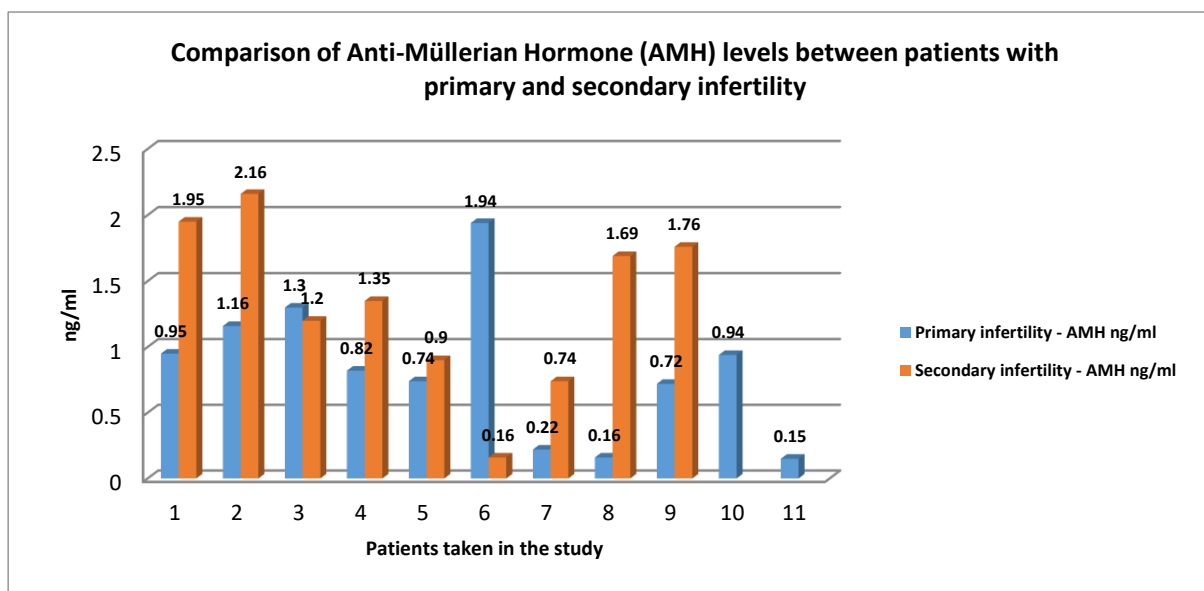
Figure 5: **Optical microscope with LED illumination with premium optics** (Humascop premium-Albimedica laboratory, 2025)

A semen analysis is a laboratory test that examines a sample of semen under a microscope. It assesses:

- How many sperm are in the seminal fluid
- The activity of the sperm (motility)
- The shape of the sperm (morphology).

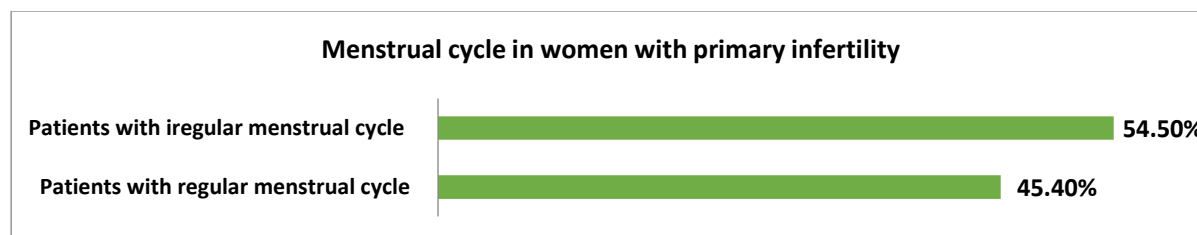
3. Results

Based on the obtained results for the Anti-müllerian hormone, Testosterone, Vitamine D, spermiogram in men analysis and infertility in different genders and human age groups. We have presented them in the graphics below.



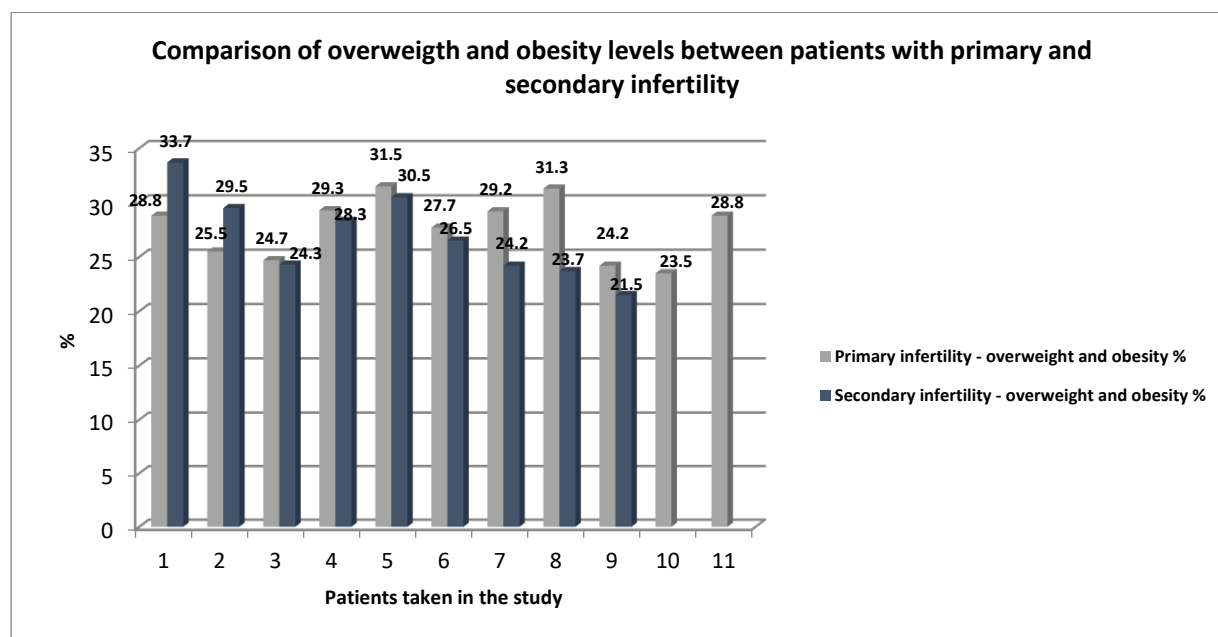
Graphic 1. AMH in women with primary and secondary infertility:

This graphic illustrates serum AMH (Anti-Müllerian Hormone) levels in women diagnosed with primary and secondary infertility. Notably, women with secondary infertility tend to show higher AMH concentrations, suggesting relatively preserved ovarian reserve compared to those with primary infertility. These findings support the role of AMH as a useful biomarker in differentiating ovarian function and guiding fertility treatment strategies.



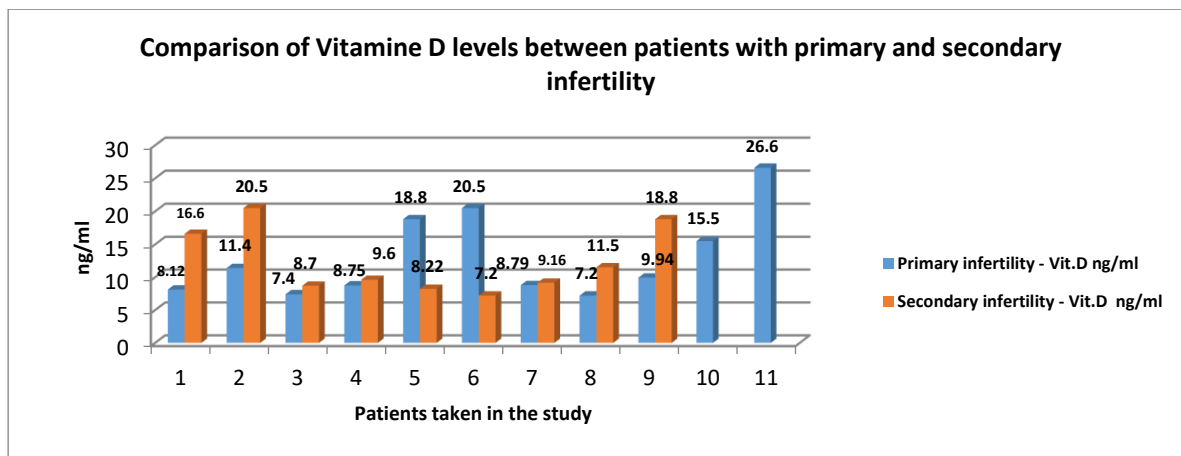
Graphic 2. Menstrual cycle in women with primary infertility:

The graphic highlights menstrual cycle irregularities in women with primary infertility. Many patients experience irregular or absent cycles, which may reflect underlying hormonal imbalances such as PCOS or anovulation. These disruptions can significantly affect fertility and should be a key focus during assessment and treatment planning.



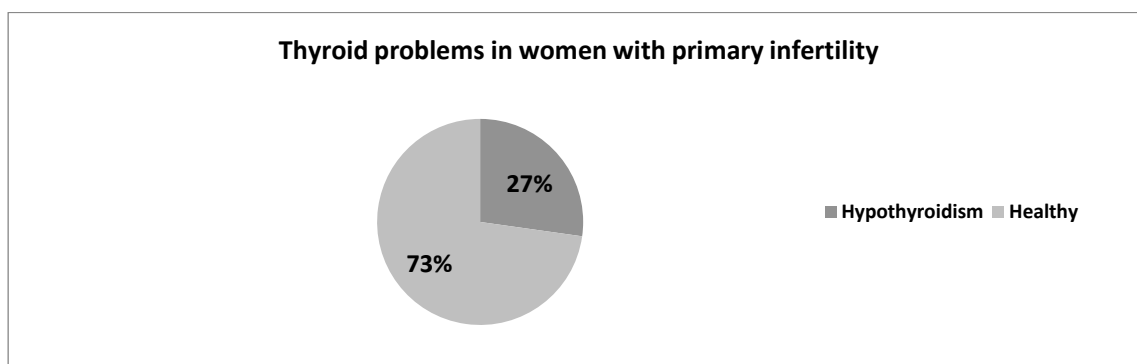
Graphic 3. Overweight - obesity in women with primary and secondary infertility:

This graphic compares the prevalence of overweight and obesity among women with primary versus secondary infertility. The data indicate a higher incidence of elevated BMI in women with secondary infertility, highlighting the potential impact of weight-related metabolic factors on subsequent fertility. These results underscore the importance of addressing lifestyle and weight management in fertility evaluations and interventions.



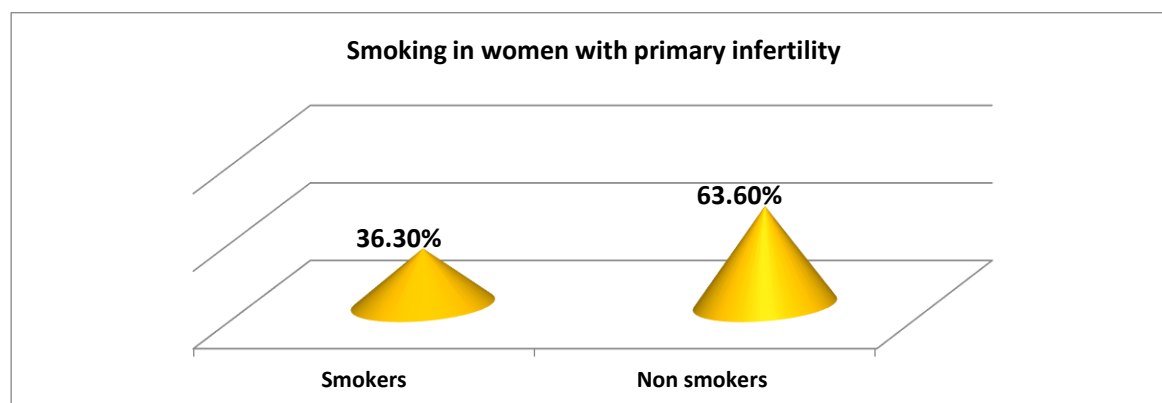
Graphic 4. Vitamin D in women with primary and secondary infertility:

This graphic presents serum Vitamin D levels in women with primary and secondary infertility. The observed differences suggest that Vitamin D deficiency may be more prevalent in one group, potentially influencing reproductive outcomes. Given Vitamin D's role in ovarian function, endometrial receptivity and hormonal regulation, these findings highlight the need to consider Vitamin D status in the assessment and management of infertility.

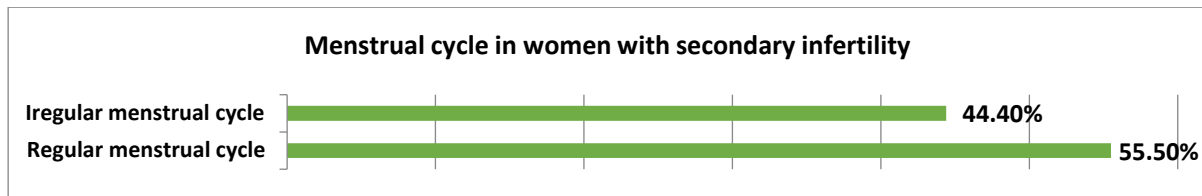


Graphic 5. Thyroid problems in women with primary infertility:

The graphic reveals a notable prevalence of thyroid disorders in women with primary infertility. Both hypothyroidism and hyperthyroidism can disrupt menstrual cycles and ovulation, contributing to fertility issues. Thyroid function should be routinely evaluated in infertility assessments.

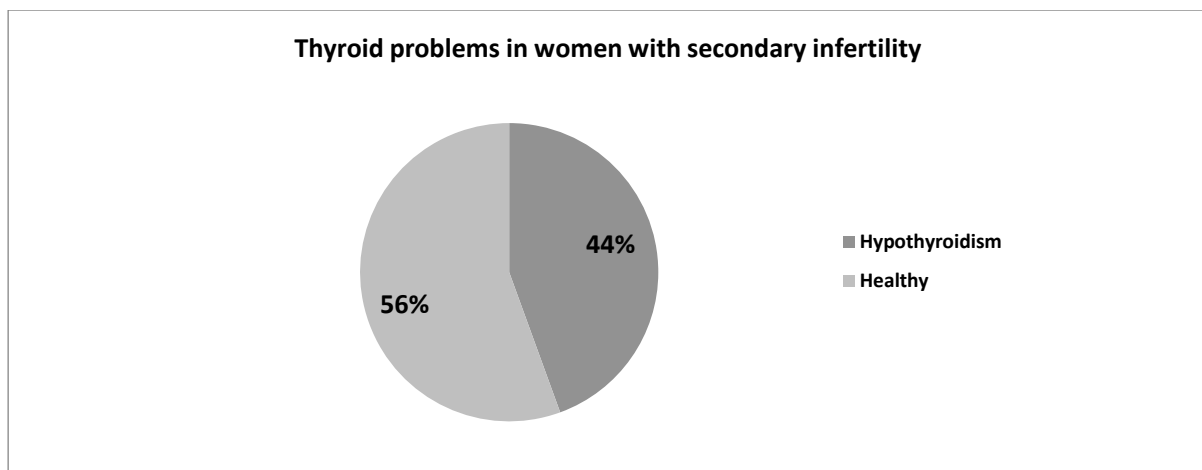


Graphic 6. Smoking in women with primary infertility: The graph shows a considerable percentage of women with primary infertility who smoke. Smoking is known to negatively affect ovarian function, hormone levels, and overall fertility. Reducing or quitting smoking is essential for improving reproductive health.



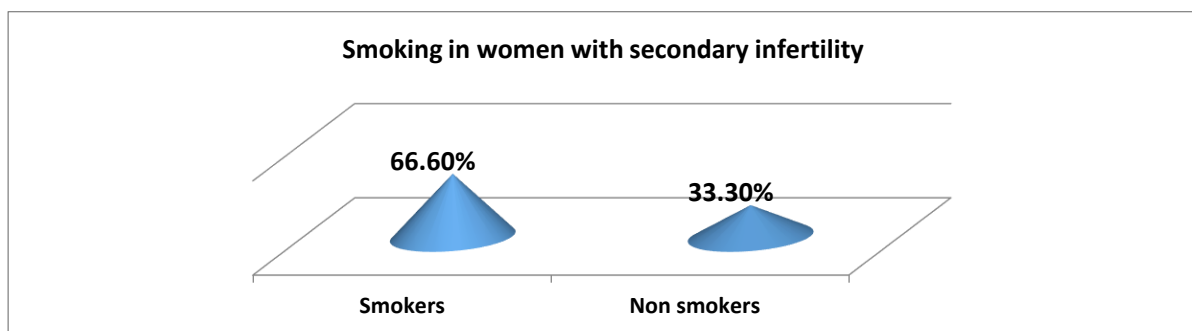
Graphic 7. Menstrual cycle in women with secondary infertility:

The graphic shows menstrual cycle irregularities in women with secondary infertility, with many experiencing anovulation or irregular cycles. These disruptions can significantly impact the ability to conceive again, highlighting the need for thorough evaluation and treatment of menstrual health in women facing secondary infertility.



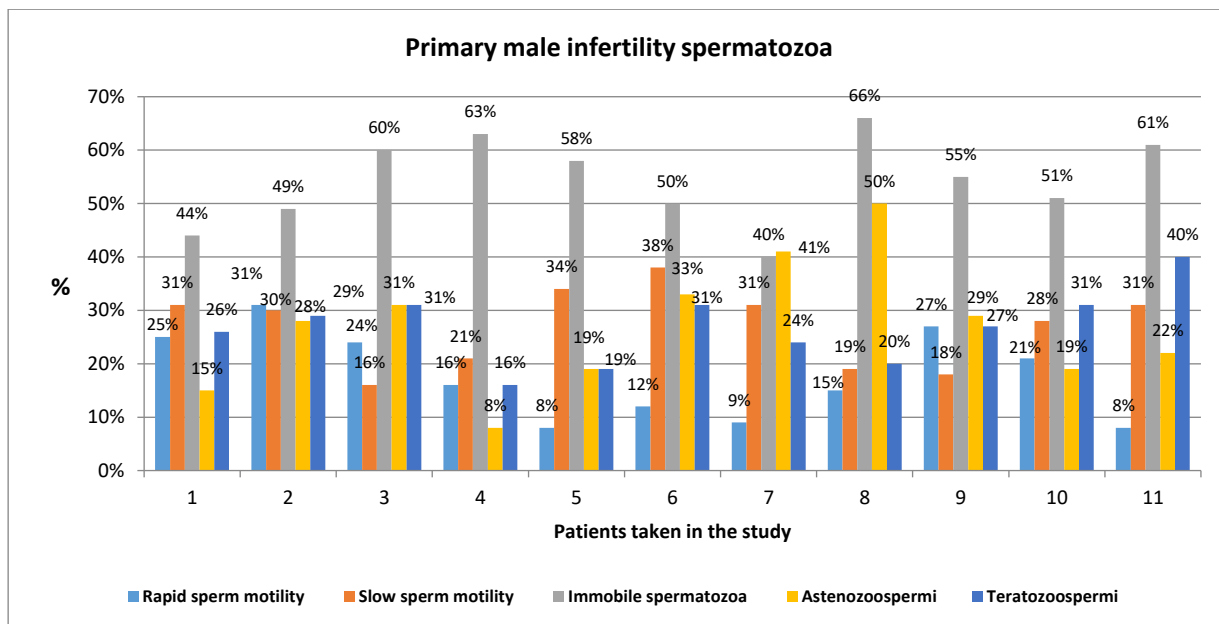
Graphic 8. Thyroid problems in women with secondary infertility:

The graphic shows a significant proportion of women with secondary infertility experiencing thyroid disorders. Both hypothyroidism and hyperthyroidism can disrupt menstrual cycles and ovulation, contributing to difficulties in conceiving again. Thyroid function should be carefully evaluated and managed to improve fertility outcomes.



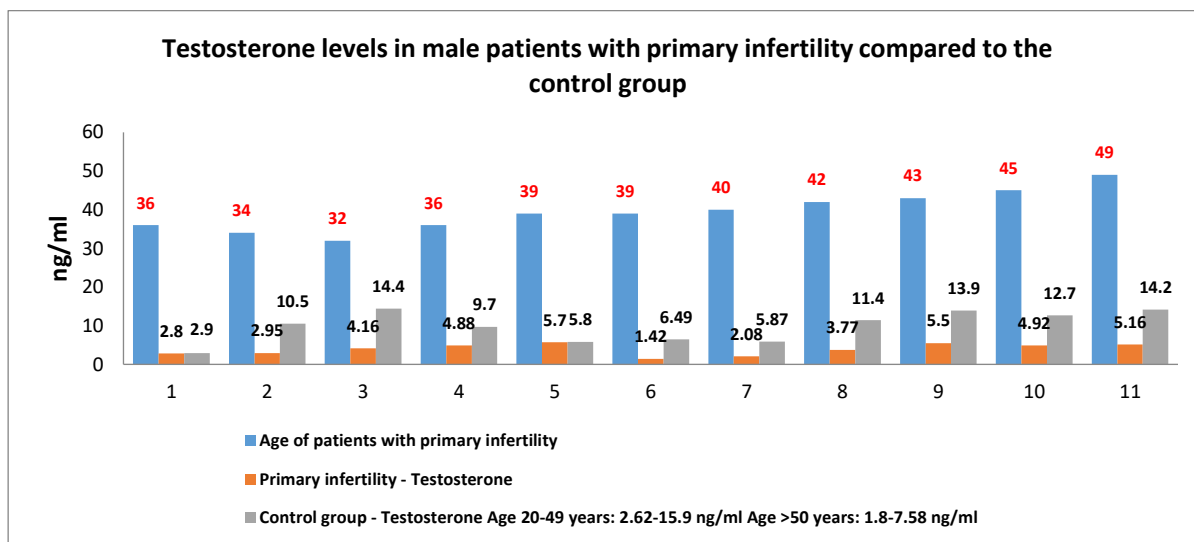
Graphic 9. Smoking in women with secondary infertility:

The graphic reveals a notable percentage of women with secondary infertility who smoke. Smoking can negatively affect ovarian reserve, egg quality, and overall reproductive health, making it harder to conceive again. Quitting smoking could significantly improve fertility outcomes in these women.



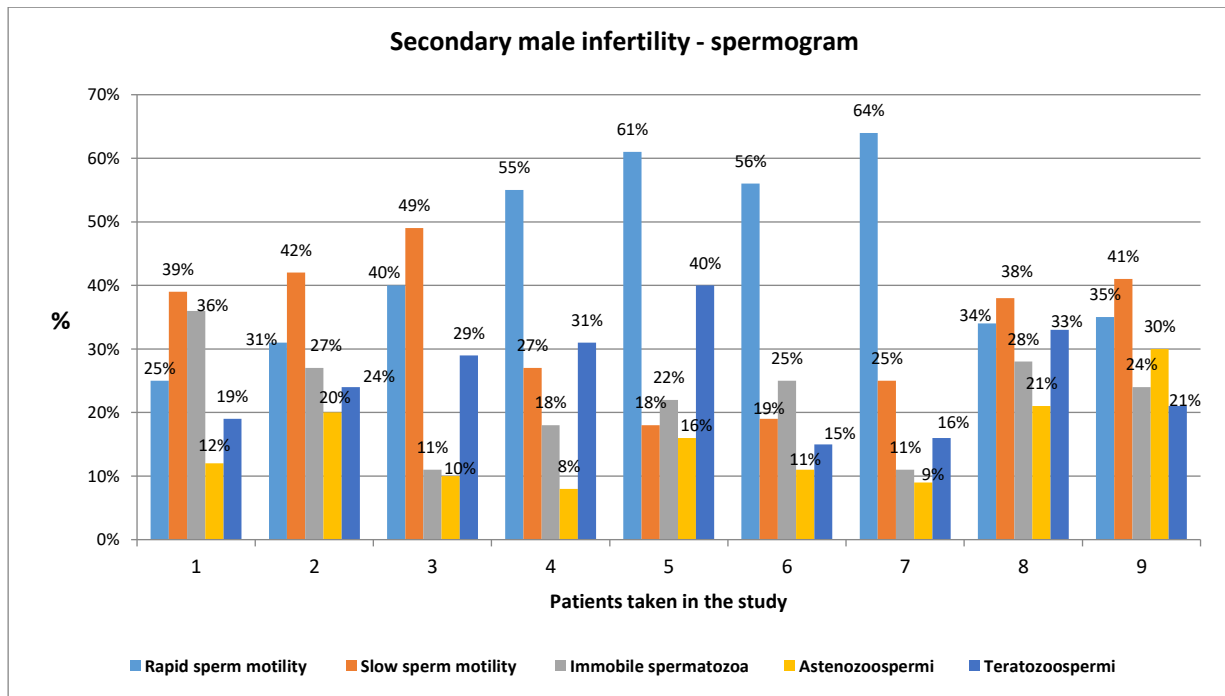
Graphic 10. Primary male infertility patient data:

In primary male infertility, reduced sperm motility is a key concern-with high rates of *asthenozoospermia* (low motility) and *teratozoospermia* (abnormal morphology). A notable proportion of patients show increased slow or immotile sperm, while rapid progressive motility is significantly impaired, directly impacting fertilization potential.



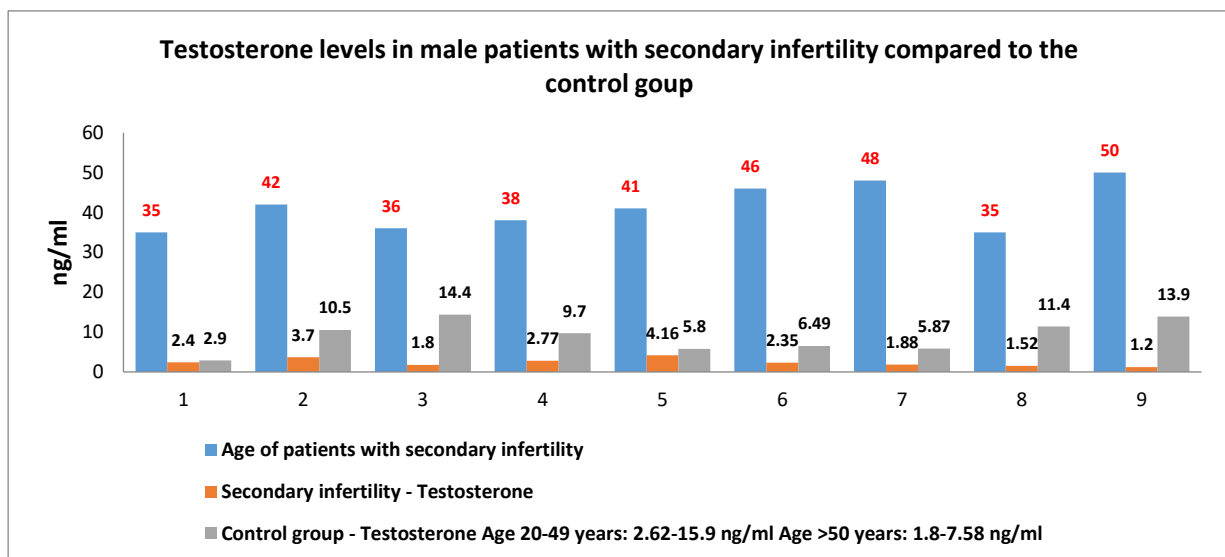
Graphic 11. Testosterone levels in male patients with primary infertility compared to the control group:

Men with primary infertility show significantly lower testosterone levels compared to the control group, highlighting a potential link between hormonal imbalance and impaired spermatogenesis. This underscores the importance of evaluating endocrine function in infertility assessments.



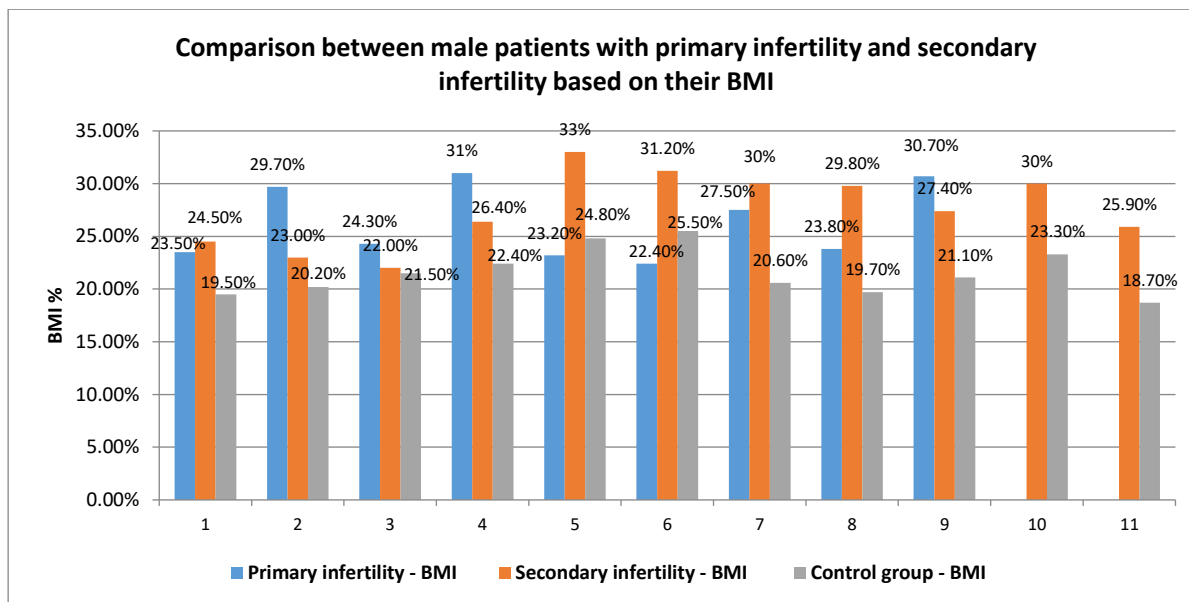
Graphic 12. Secondary male infertility patient data:

In secondary male infertility, declines in sperm quality are evident particularly in *asthenozoospermia* and *teratozoospermia*. Reduced rapid motility and a rise in slow or immotile sperm suggest progressive deterioration, even in men with prior fertility.



Graphic 13. Testosterone levels in male patients with secondary infertility compared to the control group:

In secondary infertility, testosterone levels are often moderately reduced compared to the control group, suggesting age-related or acquired factors may contribute to declining hormonal function and fertility over time.



Graphic 14. Comparison between male patients with primary infertility and secondary infertility based on their BMI:

Body mass index tends to be higher in men with secondary infertility compared to those with primary infertility, suggesting that weight gain over time may contribute to declining reproductive function in previously fertile men.

4. Discussions

Anti-Müllerian hormone (AMH) plasma levels reflect the continuous non-cyclic growth of small follicles, thereby mirroring the size of the resting primordial follicle pool and thus acting as a useful marker of ovarian reserve. Anti-Müllerian hormone seems to be the best endocrine marker for assessing the age-related decline of the ovarian pool in healthy women; thus, it has a potential ability to predict future reproductive lifespan. As a marker of ovarian reserve, AMH may be useful both to estimate the reproductive lifespan of healthy young women and to predict the ovarian response to stimulation for in vitro fertilization, namely poor and hyper-responses (Anna Garcia et al., 2012).

Women with high AMH, and Polycystic Ovary Syndrome, are best treated with weight reduction. Women with low AMH and hypogonadotropic hypogonadism should have the underlying cause corrected if possible: hyperprolactinemia, hypothyroidism, low body weight, or excessive exercise (Cedars M. I., 2022).

The relationship between AMH and Body mass index (BMI) in reproductive-aged women remains inconclusive. Research in this area is currently limited by failure to analyse the full spectrum of obesity. As markers of ovarian reserve are used to predict the value of therapies such as controlled ovarian hyperstimulation, the utility of AMH as a marker of ovarian reserve or response in women with obesity is uncertain. This uncertainty stems from the mixed results regarding the AMH and BMI relationship, as well as what a potential negative relationship could represent, whether it be truly diminished ovarian reserve or AMH as a poor proxy in populations with obesity (Kloos J et al., 2022).

According to world statistics, the prevalence of infertility among couples is 15% (Salas-Huetos A et al., 2018). Most of the pregnancies occur in the first six cycles with intercourse in the fertile phase (80%). After that, serious subfertility must be assumed in every second couple (10%) although—after 12 unsuccessful cycles untreated live birth rates among them will reach nearly

55% in the next 36 months. Thereafter (48 months), 5% of the couples are definitive infertile with a nearly zero chance of becoming spontaneously pregnant in the future (Gnoth, C., 2005). The rate of male factor infertility has been growing faster than that of female factor infertility in recent decades (Pramanik P et al., 2012). Semen quality is one of the most important determinants of infertility in men. The prevalence of infertility in men has been increasing due to the decline in semen quality in recent decades (Geoffroy-Siraudin C et al., 2012). The results of recent studies confirm a decrease in semen quality which has led to an increased willingness to conduct research on the effect of lifestyle on male fertility (Barazani Y et al., 2014). Inappropriate lifestyles have detrimental effects on sperm quality (Braga DPDAF, et al., 2012) and consequently male fertility (Sharma R et al., 2013) so that lifestyle change can improve spermogram parameters (Magdi Y et al., 2017).

Testosterone has a variety of functions and is commonly used in older men to treat symptoms of hypogonadism, such as decreased libido, decreased mood and erectile dysfunction. Despite its positive effects on sexual function, it has a negative effect on fertility. Testosterone therapy is a contraceptive, albeit a poor one. Men of reproductive age with low testosterone should be counseled on the adverse effects of Testosterone replacement therapy (TRT) on fertility. Obtaining a semen analysis and possible cryopreservation of sperm should be offered if TRT is prescribed to men interested in preserving fertility (Patel AS et al., 2019).

Vitamin D is a fat soluble vitamin that functions to maintain calcium and phosphorus homeostasis and promote bone mineralization (Institute of Medicine. Dietary Reference Intakes for calcium and vitamin D. Washington, DC: National Academy Press; 2011). Biological actions of vitamin D are mediated through vitamin D receptors (VDR) that are distributed across various tissues (Shahbazi M et al., 2011), (Zarnani AH et al., 2010), (Parikh G et al., 2010), (Blomberg Jensen M et al., 2010). The presence of VDR in reproductive tissues in both males and females suggests a possible role in the regulation of reproductive processes.

Fung JL et al., study reports a positive association between total vitamin D intake and the chance of conceiving a clinical pregnancy or live birth. When examining the intakes of the female and male together, the results continued to reveal the trend of lower clinical pregnancy rates among those couples who did not consume intakes that met the vitamin D. In addition, intake and serum levels from both the male and female partners together should be explored to determine the impact of conceiving a clinical pregnancy, and to determine if certain groups would benefit from higher vitamin D supplementation to attain optimal intakes (Fung JL et al., 2017).

5. Conclusions

Infertility is a complex condition that requires thorough evaluation. Anti-Müllerian Hormone (AMH) is a key marker of ovarian reserve, especially important in assisted reproduction. Low AMH levels are linked to poor ovarian response and early or acquired infertility, emphasizing the need for early screening and personalized care.

In men, semen analysis assessing sperm count, motility and shape is central to diagnosis and influenced by hormonal and environmental factors. Lifestyle choices like smoking harm fertility in both sexes, causing oxidative stress, DNA damage and ovarian decline. According to the latest statistics and the results obtained from the patients' spermograms, we conclude that the number of sperm in the seminal fluid is decreasing and also the quality of the sperm is decreasing due to the appearance of teratozoospermia and asthenozoospermia, which is with an increasing prevalence. Endocrine disorders such as hypothyroidism and iron-deficiency anemia disrupt ovulation and endometrial function. Obesity further impairs fertility by affecting hormones and reducing gamete quality. A comprehensive assessment of AMH, semen parameters, lifestyle, and systemic health is crucial. Future research should explore how these factors interact to guide tailored treatments. It is preferable for couples planning to have a child

after a year of unprotected intercourse to have their hormones analyzed in general, and in particular the Anti-Müllerian hormone and the spermogram of men to determine the number of spermatozoa and their quality in order to prevent infertility and in particular the necessary measures, which are very costly, for in vitro fertilization.

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