The Egyptian Journal of Hospital Medicine (April 2023) Vol. 91, Page 4487-4491

Diagnosis of Infertility: What Is New?: Review Article

Ashraf Mohamed Naser, Reda Abdel Aziz Ahmed, Wafa Khalid Ali Aeboudah, Mohamed Ahmed Helmy
Department of Obstetrics and Gynecology, Faculty of Medicine, Zagazig University, Egypt
***Corresponding author:** Wafa Khalid Ali Aeboudah, **Mobile:** (+20) 01095360339, **E-mail:** wafakhaledabouda@gmail.com

ABSTRACT

Background: One of the most common chronic health conditions affecting young adults is infertility, which is defined as a year of unsuccessful attempts at conception. The diagnosis has made progress of reproductive problems over the past ten years. **Objective:** The standard testing used to identify infertility is included in this review. We also go into other tests, such as ovarian reserve evaluation, and the prospective application of laparoscopy in the evaluation of infertility that cannot be explained.

Methods: PubMed, Google Scholar, and Science Direct were some of the places we explored for information about Infertility, Standard tests and New tests for Diagnosis. Between July 1992 and January 2022, however, only the latest or most comprehensive study was considered. The authors also assessed the usefulness of references taken from similar books. We haven't paid attention to non-English documents because we don't have the time or money to translate them. Unpublished articles, oral presentations, conference abstracts, and doctoral dissertations were all widely acknowledged to not constitute valid scientific research.

Conclusion: A thorough but time-efficient investigation of the infertile couple is required prior to a diagnosis of unexplained infertility. Couples should undergo a semen analysis, ovulation testing, assessment of ovarian reserve, and imaging to assess for tubal and uterine factors before a diagnosis of unexplained infertility is made. This workup can be completed within 1 menstrual cycle.

INTRODUCTION

Because it affects a pair rather than just one person, infertility is a rare medical illness. For women under the age of 35, it is defined as the couple's failure to conceive after 12 months of regular sexual activity without the use of contraception, and after 6 months for women 35 and older. To characterize this inability to conceive until the pair has been shown to be sterile, some professionals use the term subfertility. Fecundability, which measures the likelihood of becoming pregnant within one menstrual cycle, is a more appropriate term because it takes into account different levels of infertility **(1).**

Prevalence of Infertility:

From 2006 to 2010, the prevalence of infertility decreased from 8.5% to 6.0%. This number is lower than the incidence of infertility, which is estimated from prospective research and ranges from 12 to 18% in the United States. Additionally, it is lower than the primary infertility rate for married nulliparous women. Between 7.3 and 9.1% of married women aged 15 to 34, 25% of those aged 35 to 39, and 30% of those aged 40 to 44 experienced primary infertility. **(2).**

Investigations:

- **Semen analysis:** The main laboratory evaluation of a male partner in an infertile pair is a semen analysis. The typical semen assay comprises measurements of volume, pH, sperm motility, count, morphology, debris, and agglutination as well as leukocyte count and immature germ cells. After two to seven days have elapsed with no ejaculatory activity, the semen sample should be taken. The patient should obtain the sample while masturbating in the doctor's office, if at all possible. The sample can be collected at home and brought to the lab within an hour if it is not practical of being taken. At least two samples should be taken at least one week apart due to the significant intrinsic fluctuation of sperm concentrations in semen samples. The World Health To carry out the recommended standard procedures for World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen should be utilized while analyzing human sperm **(3).**

Lower reference levels for semen analysis have been published by the WHO. The traits listed below were discovered from a research of more than 1900 guys whose partners had a time to conception of less than 12 months, and they represent the frequently accepted 5th percentile (lower reference limits and 95% CIs are in parenthesis) **(4).**

Volume: 1.5 mL (95% CI 1.4-1.7)

- **Sperm concentration**: 15 million spermatozoa per milliliter (95% CI: 12–16)
- **Total sperm number**: 95% confidence interval: 33–46; 39 million spermatozoa per ejaculate
- **Morphology**: employing the "strict" Tygerberg technique, 4% of the normal forms (95% CI 3-4).
- **Vitality**: 95% confidence interval: 55-63)
- **Progressive motility**: (99% confidence interval: 31-34)
- **Total (progressive+ non-progressive) motility**: 40% (95% CI: 38-42)
- **Additional evaluation**: Men with infertility should go through the following evaluations following the

Received: 18/11/2022
Accepted: 20/01/2023

preliminary assessment (history, physical exam, and two analyses of semen).

- **Men with a normal semen analysis**: The couple should think about being sent to an expert in ART, following the female partner's participation in in vitro fertilization (IVF) has undergone a thorough examination and has received therapy for reversible causes of female infertility [5].

- **Men with an abnormal semen analysis:**
- **Normal sperm concentration, abnormal morphology and/or motility**: A recommendation to an ART expert who specializes in intracytoplasmic sperm injection (ICSI), may be beneficial [5].
- **Sperm concentration < 10 million/mL**: Men with infertility and sperm concentrations under 10 million/mL should have their measurements of the blood levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone because Klinefelter syndrome is common in this group of men [6].
- **Severe oligozoospermia or azoospermia**: Genetic testing is necessary for men who have extreme azoospermia or oligozoospermia in addition to checking for hormones. For the examination of obstructive azoospermia in some men transrectal ultrasound may be required in these patients (those with testicular volume normal, visible vasa deferentia on inspection, normal endocrine tests, and azoospermia) [5].

- **Endocrine testing**: An infertile male with a poor sperm concentration's endocrine evaluation (<10 million/mL) involves assessments serum LH, FSH, and total testosterone as well as additional assays as determined by clinical need [7].
- **Low testosterone, and high FSH and LH**: Leydig cell function and primary (hypergonadotropic) hypogonadism are both impacted. A karyotype should be conducted on these guys.
- **Normal testosterone and LH, and high FSH**: Primary (hypergonadotropic) hypogonadism is characterized by seminiferous tubule destruction without Leydig cell failure.

- **Low testosterone, but FSH and LH not elevated (normal or low)**: Hypogonadism that is secondary (hypogonadotropic). Men with low serum testosterone levels and normal to low serum LH levels should have their serum prolactin levels checked. Some males could require further testing for secondary hypothyroidism, secondary hypoadrenalism, and a sellar tumor.
- **High testosterone and LH, but normal FSH**: partial resistance to androgen.
- **Normal testosterone, LH, and FSH**: The results of semen analysis, such as azoospermia, oligozoospermia, asthenozoospermia, or teratozoospermia, will determine whether further study is warranted. Men who have azoospermia and normal endocrine testing should have their ejaculatory ducts examined.
- **Low sperm count and very low LH in a man who is very muscular**: suspect of abusing androgens.
- **Scrotal and transrectal ultrasound**: The most likely diagnosis is obstructive azoospermia if a patient exhibits Vasa deferentia perceptible on examination, azoospermia, and normal serum levels of testosterone, FSH, and LH. An ultrasound of the transrectal or scrotal region that shows dilated seminal vesicles can detect ejaculatory duct occlusion [8].

- **Genetic tests**: Genetic testing may involve karyotyping, testing for Y-chromosome microdeletions, or testing for mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), depending on the patient's clinical presentation. ICSI has made it possible for men with severe oligozoospermia and azoospermia to have children can now become parents, however there are significant genetic risks connected to this invasive treatment. These risks include Y chromosomal microdeletions, anomalies of the somatic and sex chromosomes, transmission of the CFTR gene, X chromosome issues, and epigenetic effects on the child [9].

- **Chromosomal anomalies**: For infertile guys with increased blood FSH and LH concentrations and sperm counts less than 10 million/mL, kytotyping is advised. A majority frequent abnormality of the sex chromosome is Klinefelter syndrome. Usually, the testes of these males are tiny and solid [10].
- **Evaluation of female infertility:**
- **History:** The most significant historical events include [11]:

Duration of infertility and the outcomes of earlier testing and treatment.

- **Menstrual history** (cycle duration and features), which aids in figuring out whether or not one is ovulating. For instance, symptoms like severe dysmenorrhea suggest endometriosis, while normal. The patient is ovulatory when they have Molimina (breast soreness, ovulatory discomfort, and bloating) throughout monthly periods.
- **Medical, surgical, and gynecological history** (such as treatment for abnormal Pap smears, pelvic inflammatory disease, and STDs) to search for illnesses, treatments, or drugs that might be connected to infertility. The assessment of the patient's systems must at the very least reveal whether they exhibit symptoms of dyspareunia, dysmenorrhea, hirsutism, pelvic or abdominal discomfort, and galactorrhea. A prior unilateral oophorectomy may affect fertility in older women because they may experience diminished ovarian reserve earlier than those who have two ovaries. Young

women who have undergone unilateral oophorectomy typically do not have reduced fertility because they have many primordial follicles per ovary.

- **Obstetrical history** to look for circumstances that might be linked to future infertility or unfavorable pregnancy outcomes.
- **Sexual history**, including coitus frequency and sexual dysfunction. Infertility may have a biological cause, such as infrequent or poor coitus.
- **Family history**, including members of the family who are infertile, have birth deformities, have genetic mutations, or are mentally retarded. Males with the fragile X premutation may have learning difficulties, developmental delays, or autistic traits, while women may experience premature ovarian failure.
- **Personal and lifestyle history** among the factors that can affect fertility include age, profession, physical activity, stress, diets and weight swings, smoking, and alcohol use.
- **Physical examination:** Indicators of potential causes of infertility should be looked for during the physical examination [(11)].
- It is important to determine the body mass index (BMI) of the patient and take note of their fat distribution because BMI extremes are linked to decreased fertility and abdominal obesity is linked to insulin resistance.
- Hypogonadotropic hypogonadism is indicated by incomplete emergence of secondary sexual traits while there is primary amenorrhea. In patients with absent periods, a short, stocky body type with a square-shaped chest is suggestive of Turner syndrome.
- Endocrinopathies (such as hyper- or hypothyroidism, hyperprolactinemia, polycystic ovarian syndrome, adrenal problem) may be present if there are the emergence of secondary sexual traits while there is primary amenorrhea.
- Adnexal or posterior cul-de-sac lumps or tenderness are indicative of endometriosis or chronic pelvic inflammatory illness. Additional indications of endometriosis include palpable sensitive nodules in the rectovaginal septum, uterosacral ligaments, or posterior cul-de-sac.
- Discharge or structural anomalies indicate the existence of an infection, a cervical factor, or a müllerian abnormality in the vagina or cervical region.
- Enlargement, irregularity, and or immobility are symptoms of pelvic adhesive disease, leiomyoma, endometriosis, and uterine anomalies.

Diagnostic tests:

Assessment of ovulatory function:

- **Mid-luteal phase serum progesterone level:** Around a week prior to the anticipated menstrual cycle, this needs to be obtained. The test would be conducted on day 21 of a typical 28-day cycle, level of progesterone >3 ng/mL is evidence of recent ovulation. If the mid-luteal progesterone concentration is <3 ng/mL, the patient is considered when evaluating anovulation-related issues. TSH, FSH, serum prolactin, and a test for polycystic ovarian syndrome (PCOS) are all part of the minimal work-up [(12)].
- **LH surge:** A urine ovulation prediction kit sold without a prescription is used by the patient. The luteinizing hormone (LH) that reliably signals ovulation may be detected by these kits, which are also quite good at forecasting when the LH spike will occur. The false positive and false negative rate for home kits is between 5 and 10%. As a result, patients who are unable to identify a urine LH spike may benefit from serum confirmation [(11)].
- **Folliculometry:** Daily ultrasounds to track a follicle's growth and eventual elimination (the most precise way to track ovulation) [(13)].
- **Endometrial biopsy:** for routine diagnostic evaluation of ovulation are too expensive or intrusive to detect secretory changes in the endometrium. [(13)].

- **Assessment of ovarian reserve:**

We advise assessing the ovarian for women under 35 and those over 35 who have risk factors for early ovarian failure, a day 3 FSH and estradiol level reserve. Some specialists and in particular situations use additional tests such the clomiphene citrate challenge test (CCCT), antral follicle count, and AMH level. The value of these tests in determining whether IVF cycles will be successful is limited, but their specificity for predicting a poor response [(14)].

- **Day 3 FSH and CCCT:** On cycle days 5 through 9, 100 mg of clomiphene citrate is administered orally, and the levels of day 3 estradiol and day 3 FSH are measured. An acceptable ovarian reserve is indicated by a day 3 FSH concentration of less than 10 mIU/mL, with levels of 10 to 15 mIU/mL being borderline. Because different FSH assay reference standards and testing protocols are used, cutoff values of 10: 25 mIU/mL have been noted as the maximum value for a typical FSH concentration. Levels of estradiol on cycle day 3 of 80 pg/mL are a sign that the ovarian reserve is sufficient. Advanced premature follicle recruitment, which happens in women with limited ovarian reserve, causes elevated baseline estradiol levels. Estradiol can stop the pituitary from producing FSH at high doses, concealing one of perimenopausal women's signs of a lowered ovarian reserve. In order to prevent false-negative FSH testing, it is therefore helpful to evaluate both FSH and estradiol levels [(15)].
- **Antral follicle count (AFC):** Antral follicles are described as follicles that range in size from 2 to 10 mm. The number of these follicles can be counted via ultrasound examination. a minimal AFC varying from

<4 to 10, a low ovarian reserve is indicated by the presence of between days two and four of a typical menstrual cycle, antral follicles. AFC has a decent oocyte quality, however it has a lower predictive value for ovarian reserve and responsiveness the success of IVF, and the fate of pregnancies [16].

- **Anti-müllerian hormone (AMH):** The small intestine produces AMH, which is a TGF-beta family member and the anti-müllerian hormone (<8 mm) is indicator to follicles that are preantral and early antral. In a variety of clinical circumstances, the AMH level may be the most significant biochemical indicator of ovarian function since it indicates the size of the primordial follicle pool. Adult women's AMH levels gradually decrease as the primordial follicle pool ages; by menopause, AMH is undetectable [17]. One review suggested the general rules listed below [18]:
- **AMH <0.5 ng/mL** predicts decreased ovarian reserve in a cycle of IVF with less than three follicles.
- **AMH <1.0 ng/mL** links the chance of having few eggs to baseline ovarian reserve being retrieved.
- **AMH >1.0 ng/mL but <3.5 ng/mL** demonstrates a positive response to stimuli.
- **AMH >3.5 ng/mL** is likely to have a strong reaction to ovarian stimulation, hence to prevent ovarian hyperstimulation syndrome, caution should be exercised.
- Unlike day 3 FSH, AMH can be detected at any point due to the ongoing cycle, the menstrual cycle frequently exhibits little intracycle and intercycle fluctuation rather than cyclical growth of the tiny preantral follicles that express it. [19].

Role of laparoscopy:

Laparoscopy's role in the assessment of infertility is debatable. Costly and invasive, laparoscopy. The results of the laparoscopy often if the initial infertility screening is negative or reveals severe male factor infertility, do not change the infertile couple's initial course of treatment. As part of the workup for women who present for the evaluation of infertility, the doctor must determine whether to perform surgical exploration for endometriosis and other disorders because endometriosis may be present in up to 50% of women who complain of infertility [20].

Laparoscopy is advised for women based on physical examination, HSG, or history (such as current dysmenorrhea, pelvic discomfort, or deep dyspareunia; prior acute appendicitis, pelvic infection, pelvic surgery, or ectopic pregnancy) and endometriosis or pelvic adhesions/tubal disease that may be present. Along with laparoscopy, we also do hysteroscopy to examine the uterine cavity and chromotubation to determine tubal patency. Because of this, if a laparoscopy is anticipated, HSG can be skipped [21].

A comprehensive infertility evaluation is performed on both the partners and the women who have infertility but no endometriosis symptoms or history of past surgery. Normal infertility evaluation couples, those with unexplained infertility, typically women experience intrauterine insemination or ovarian stimulation, and become pregnant naturally. The efficiency of diagnostic laparoscopy and when it should be performed in couples experiencing unexplained infertility before ovulation induction have not been evaluated in randomized studies. Male or tubal infertility patients are frequently given IVF as a therapeutic option instead of laparoscopy [22].

The benefit of performing a laparoscopy early in a woman's examination for the surgical treatment for endometriosis or pelvic adhesions can begin without potentially unnecessary or ineffective medical procedure to induce ovulation on an ad hoc basis. The ability to lyse pelvic adhesions and endometriosis can be removed or ablated if found during the diagnostic procedure [22].

Tests of limited clinical utility:

- **Postcoital test:** Due to disagreement about what constitutes a normal vs abnormal test result, as well as low inter- and intraobserver reliability, the postcoital test has limited diagnostic potential and predictive usefulness repeatability. Additionally, efforts to reduce infertility due to cervical factors have not been successful, and since common infertility treatments like intrauterine insemination and IVF don't involve the cervix, reducing infertility due to cervical factors is pointless [23].
- **Endometrial biopsy:** The lack of efficacy of endometrial biopsy is highlighted by using the American Society of Reproductive Medicine to evaluate women who are infertile and discourages its usage absent a strong suspicion of endometrial disease [23].
- **Basal body temperature records:** The cheapest approach for determining ovulation is basal body temperature charts, although interpreting the charts can be challenging and vulnerable to significant interobserver variance [24].
- **Zona-free hamster oocyte penetration test:** The sperm penetration assay is another name for this test. On whether the hamster oocyte, there is mixed evidence in the literature, however the test predicts human oocyte fertilization. The proficiency of the laboratory performing the assay has an impact on the utility of test results. Because the findings wouldn't have an impact on our clinical management, we do not order this test [25].
- **Mycoplasma cultures:** Getting accustomed to Mycoplasma hominis and Urea plasma urealyticum cultures are not recommended because there is scant evidence linking these organisms to female infertility [26].

- **Testing for antibodies:** Existing research does not support regular testing for thyroid, thyroid hormone, antisperm, and antinuclear antibodies. Although recurrent pregnancy loss and antiphospholipid antibodies have been linked, other autoimmune variables are still being researched as potential indicators of fertility therapy failure [(27)].
- **Karyotype:** There is a general consensus that if there is significant oligospermia, counsel the male spouse and offer to karyotype him because these individuals are more likely to have karyotypic anomalies. There may also be a separate test for Y chromosomal microdeletions available. If there have been repeated miscarriages, karyotyping is advised for both partners and women who have very early menopause (before to age 40). Due to the low occurrence of anomalies, infertility caused by the tubal factor, endometriosis, or other causes in females karyotyping is typically not recommended as part of the first evaluation in other situations [(28)].

Financial support: No specific grant was given to this research by any funding organization in the public, private, or nonprofit sectors.

Conflict of interest: The writers affirmed that they have no financial or other conflicts of interest.

REFERENCES

1. **Practice Committee of tAmerican Society for Reproductive Medicine (2008):** Definitions of infertility and recurrent pregnancy loss. Fertil Steril., 90: S60-S62.
2. **Thoma E, McLain C, Louis F *et al.* (2013):** Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. Fertil Steril., 99: 15-17.
3. **Wang C, Swerdloff S (2014):** Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. Fertility and sterility, 102 (6): 1502-1507.
4. **Cooper G, Noonan E, von Eckardstein S *et al.* (2010):** World Health Organization reference values for human semen characteristics. Hum Reprod Update, 16: 231-239.
5. **Anawalt D, Page T, Matsumoto M *et al.* (2019):** Causes of male infertility. https://www.medilib.ir/uptodate/show/7473.
6. **Hofherr E, Wiktor E, Kipp R *et al.* (2011):** Clinical diagnostic testing for the cytogenetic and molecular causes of male infertility: the Mayo Clinic experience. J Assist Reprod Genet., 28: 91-99.
7. **Anawalt D (2013):** The silent spermatozoon: are man-made endocrine disruptors killing male fertility? Asian J Androl., 15: 165-169.
8. **Abdulwahed R, Mohamed E, Taha A *et al.* (2013):** Sensitivity and specificity of ultrasonography in predicting etiology of azoospermia. Urology, 81: 9 67-969.
9. **Hotaling J, Carrell T (2014):** Clinical genetic testing for male factor infertility: current applications and future directions. Andrology, 2:3 39-342.
10. **McLachlan I, O'Bryan K (2010):** Clinical Review#: State of the art for genetic testing of infertile men. J Clin Endocrinol Metab., 95: 1013-1018.
11. **Kuohung W, Hornstein D, Barbieri L *et al.* (2019):** Evaluation of female infertility. https://medilib.ir/uptodate/show/5445
12. **Wathen C, Perry L, Lilford J *et al*. (1984):** Interpretation of single progesterone measurement in diagnosis of anovulation and defective luteal phase: observations on analysis of the normal range. Br Med J., 7: 55-59.
13. **Ecochard R, Boehringer H, Rabilloud M *et al.* (2019):** Chronological aspects of ultrasonic, hormonal, and other indirect indices of ovulation. BJOG., 10: 113-119.
14. **Podfigurna A, Lukaszuk K, Czyzyk A *et al.* (2018):** Testing ovarian reserve in pre-menopausal women: why, whom and how?. Maturitas, 109: 112-117.
15. **Souter I, Dimitriadis I, Baltagi M *et al.* (2014):** Elevated day 3 follicle-stimulating hormone in younger women: is gonadotropin stimulation/intrauterine insemination a good option? Am J Obstet Gynecol., 211: 62-65.
16. **Jayaprakasan K, Chan Y, Islam R *et al.* (2012):** Prediction of in vitro fertilization outcome at different antral follicle count thresholds in a prospective cohort of 1,012 women. Fertil Steril., 98: 657-659.
17. **Dewailly D, Andersen Y, Balen A *et al*. (2014):** The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update, 20: 370-377.
18. **Toner P, Seifer B (2013):** Why we may abandon basal follicle-stimulating hormone testing: a sea change in determining ovarian reserve using antimüllerian hormone. Fertil Steril., 99: 1825-1830.
19. **Dorgan F, Spittle S, Egleston L *et al.* (2010):** Assay reproducibility and within-person variation of Müllerian inhibiting substance. Fertil Steril., 94: 301-306.
20. **Kuohung W, Hornstein D, Barbieri L *et al.* (2016).** Evaluation of female infertility. https://medilib.ir/uptodate/show/5445
21. **Luttjeboer Y, Verhoeve R, van Dessel J *et al.* (2009):** The value of medical history taking as risk indicator for tuboperitoneal pathology: a systematic review. BJOG., 116: 612-614.
22. **Amer K (2015):** Laparoscopic Ovarian Drilling. Reproductive Surgery in Assisted Conception, 6: 61-71.
23. **Penzias A, Azziz R, Bendikson K (2021):** Fertility evaluation of infertile women: a committee opinion. Fertility and sterility, 116 (5): 1255-1265.
24. **Kambic R, Gray H (1989):** Interobserver variation in estimation of day of conception intercourse using selected natural family planning charts. Fertil Steril., 51: 430-433.
25. **Shibahara H, Mitsuo M, Inoue M *et al.* (2019):** Relationship between human in-vitro fertilization and intracytoplasmic sperm injection and the zona-free hamster egg penetration test. Hum Reprod., 13: 1928-1933.
26. **Gump W, Gibson M, Ashikaga T (1984):** Lack of association between genital mycoplasmas and infertility. N Engl J Med., 310: 937-947.
27. **Kallen B, Arici A (2003):** Immune testing in fertility practice: truth or deception? Curr Opin Obstet Gynecol., 15:225-230.
28. **Papanikolaou G, Vernaeve V, Kolibianakis E *et al.* (2005):** Is chromosome analysis mandatory in the initial investigation of normovulatory women seeking infertility treatment? Hum Reprod., 20: 2899-2903.