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Diagnostic workup for a couple with unexplained infertility – VVOG guidance

1. Objective of this clinical guidance paper

The primary objective of this paper is to provide clinicians (specifically general practitioners, gynecologists, andrologists and fertility specialists) with evidence-based guidance regarding couples with unexplained infertility (UI). Unexplained infertility (UI) is a diagnosis of exclusion given to couples who have been unable to conceive for one year despite standard fertility investigations failing to identify any abnormalities or a specific diagnosis [1].

More precisely, it aims to recommend the appropriate diagnostic workup before diagnosing patients with UI within the context of medical practice in Flanders. Because discussing therapeutic implications would take us too far afield, we have decided to focus solely on the diagnostic aspects of UI.

2. Summary table of recommendations

Recommendation	Grade (strength of recommendation)	Strength evidence = level of evidence
Ovarian reserve		
In women with regular menstrual cycles, the panel recommends against using ovarian reserve testing to predict the probability of spontaneous conception within 6–12 months in couples with UI.	xxx	+ +
Ovulation		
In women with regular menstrual cycles, there is no indication of routine ovulation confirmation tests.	GCP	–
If ovulation must be confirmed, ultrasound monitoring, urinary LH measurement, mid-luteal serum progesterone can be used	xxx	+
Luteal phase		
In women with regular menstrual cycles, we do not consider routinely measuring midluteal serum progesterone levels.	xxx	+ +
There is no reliable method to diagnose luteal phase deficiency.	xxx	+ +
In women investigated for infertility, endometrial biopsy for histological examination is not recommended in the absence of other indications.	xxx	+ +
Tubal factor		
Chlamydia IgG antibody testing could be used as a non-invasive test to differentiate between women at low versus high risk for tubal occlusion.	xxx	+ +
In women at high risk of tubal occlusion, based on Chlamydia antibody testing or medical history, tubal patency testing should be performed.	GCP	-
Hysterosalpingo-foam sonography (HyFoSy) and hysterosalpingography (HSG) are both valid tests for assessing tubal patency when compared to laparoscopy with chromopertubation (methylene blue).	xxxx	+ ++ +
HSG and HyFoSy are comparable in terms of diagnostic capabilities; however, HyFoSy might be preferred.	GCP	–
Uterine factor		
Ultrasound, preferably 3D-ultrasound, is recommended to exclude uterine anomalies in women with UI.	xxx	+ +
If ultrasound assessment of the uterine cavity is normal no further evaluation with hysteroscopy, HyCoSy or MRI is needed.	xxxx	+ +
Laparoscopy		
Routine laparoscopy is not recommended for the diagnosis of unexplained infertility.	xxxx	+ ++
Laparoscopy could be considered for women in whom pelvic pathology is suspected or those presenting with symptoms suggestive of endometriosis.	xxx	+ +
Male additional tests		
Testing for anti-sperm antibodies (ASA) is not recommended when semen analysis, according to WHO criteria, is normal.	xxx	+ +
DNA fragmentation test is not recommended when semen analysis according to WHO criteria is normal.	xxxx	+ +
Additional testing		
Measuring TSH is considered good practice in preconception care.	GCP	–

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Recommendation	Grade (strength of recommendation)	Strength evidence = level of evidence
No additional thyroid evaluation tests are recommended for women if TSH levels are within the normal range.	xxxx	+ +
We do not recommend treating women with high-normal TSH values (TSH 2.5–4.5 mIU/L)	xxx	+ +

Grade (strength of recommendation)	**Level of evidence**
xxxx: strong for	+ ++ +: high
xxx: weak for	+ ++ : average
xx: weak against	+ +: low
x: strong against	+ : very low
GCP: good clinical practice	−: no evidence

3. Acronyms, keywords and abbreviations

AFC Antral Follicle Count
AMH Anti-Müllerian Hormone
ART Assisted reproductive technology
ASA Anti-sperm antibodies
CAT Chlamydia antibody testing
CI Confidence interval
DFI DNA fragmentation index
FSH Follicle-stimulating hormone
HSG Hysterosalpingography
HyCoSy Hysterosalpingo-contrast sonography
HyFoSy Hysterosalpingo-foam sonography
ICSI Intracytoplasmic sperm injection
IUI Intrauterine insemination
IVF In vitro fertilization
LH Luteinizing hormone
LPD Luteal phase deficiency
OSCM Oil-soluble contrast media
OR Odds ratio
PID Pelvic inflammatory disease
RCT Randomized control trial
SDF Sperm DNA fragmentation
TSH Thyroid-stimulating hormone
T4 Thyroxine
UI Unexplained infertility
US Ultrasound
VVOG Vlaamse Vereniging voor Obstetrie en Gynaecologie

4. Background

Unexplained infertility (UI) is a diagnosis of exclusion given to couples who have been unable to conceive despite standard fertility investigations failing to identify any abnormalities or a specific diagnosis. Although it affects approximately 30 % of couples seeking fertility treatment, UI remains a controversial diagnosis, as it indicates an unexplained inability to conceive despite normal reproductive health in both partners. In many cases, the cause remains unknown despite thorough evaluation. There is still a wide variation in the criteria used to diagnose UI, as well as the necessity of ruling out other pelvic conditions, for instance the existence of (asymptomatic) peritoneal endometriosis, before classifying the infertility as unexplained [1]. This heterogeneity results in bias when comparing treatment effects reported from different randomized controlled trials (RCTs) [2].

The panel adopted the definition of UI provided by ESHRE. UI is defined as infertility in couples with apparently normal ovarian function with a regular cycle of 24–38 days, normal anatomy of the fallopian tubes, uterus and cervix, and female age of $\leq$ 40 years. Additionally, male partners must have normal testicular function, genitourinary anatomy, and normal semen parameters as per WHO criteria. Moreover, infertility is only diagnosed when couples have had at least 12 months of regular, unprotected sexual intercourse before any investigations are initiated. The concept of regular intercourse is extremely variable and particular to each couple. Applying strict bounds to define regular intercourse is unadvisable and could cause unnecessary stress in those seeking to conceive. It can be recommended to increase the frequency of sexual intercourse to at least every 2–3 days during the fertility window (5 days before ovulation plus day of ovulation) [3].

5. Evidence

Initially, a literature review was conducted to obtain the most recent international guidelines. The objective was to evaluate these guidelines and determine whether their recommendations could be extrapolated to the Flemish population and health care setting. The following databases were consulted for the literature review: PubMed, Embase, and the Cochrane Library, using a combination of the keywords ‘unexplained infertility’ AND ‘guideline’. Additionally, guidelines from prominent national and international gynecology and obstetrics associations that were not retrieved via the above search strategy were also considered. Only guidelines published after 2017 and written in Dutch or English were included.

After excluding irrelevant papers based on title and abstract, as well as guidelines that did not focus on the diagnosis of unexplained infertility, a total of six guidelines were selected. The selected guidelines were assessed using the AGREE-II instrument. Each guideline was independently evaluated by two panel members. Guidelines were included only if they received an average score greater than 60 %. These guidelines were considered reliable and assessed to have a low risk of bias.

After exclusions, five guidelines were incorporated for recommendations related to diagnostics in unexplained infertility [3–7]. For some of the recommendations, where the included guidelines lacked sufficient scientific evidence or were absent, relevant scientific articles were included to supplement these recommendations. Finally, the ADAPT framework was used to adapt the included guidelines to the Flemish health care setting. Where necessary, targeted literature searches were performed to ensure the comprehensiveness and relevance of the evidence.

6. Recommendations

6.1. Ovarian reserve

6.1.1. Should ovarian reserve tests be included in the workup for unexplained infertility?

6.1.1.1. Anti-mullerian hormone (AMH). AMH is a glycoprotein produced by developing granulosa cells of (pre) antral follicles. Its value correlates with the quantity of primordial follicles and is inversely associated with female age. Consequently, AMH reference values are age adjusted and seem to be poorly associated with pregnancy rates in natural conceptions, without reflecting oocyte quality [4].

A cohort study of 148 couples with UI suggested that lower AMH values were significantly associated with UI, whereas antral follicle count was not. Steiner et al. showed that women between 30 and 44 years with low AMH values (<0,7 ng/mL) had a similar predicted pregnancy rate by 12 cycles (84 %, 95 % CI 70–91 %) compared with women with normal AMH values (75 %, 95 % CI 70–79 %). Another cohort study of 102 women showed no predictive value of AMH, AFC and basal FSH measurements for the time to ongoing pregnancy. Similarly, in a cohort study of 186 couples attempting pregnancy over six menstrual cycles, women aged 20–35 years with the lowest quintile of AMH levels exhibited similar fecundability to women with medium

serum AMH levels. In another small cohort study by Casadei et al., pregnancy and live birth rates were similar between women with low AMH values (<0.75 ng/mL) and those with higher values. This finding suggests that AMH is a poor predictor of live birth rates over a five-year period [3].

6.1.1.2. Antral Follicle Count. The antral follicle count (AFC) is defined as the number of preantral follicles (ranging from 2 to 10 mm) in the early follicular phase (days 2–4). AFC has shown good predictive value for ovarian response to stimulation in in vitro fertilization (IVF) settings, but its predictive value for spontaneous pregnancy is low [8]. Casadei et al. demonstrated that AFC is not correlated with spontaneous pregnancy. Similar AMH and AFC values were observed in women from both infertile couples and those with no history of infertility [3].

6.1.1.3. Day 3 Follicle-Stimulating Hormone. Early follicular phase FSH (cycle day 2–5) is an indirect marker of ovarian reserve based on the negative feedback inhibition by ovarian hormones. FSH is highly specific for predicting poor ovarian response to stimulation (FSH > 10 IU/L), but its sensitivity is poor (11–86 %) [4, 8].

A large cohort study of 750 women found no difference in cumulative fecundity between women with FSH > 10 IU/L and those with normal FSH values after 12 natural cycles, with cumulative fecundity rates of 82 % (95 % CI 70–89 %) vs. 75 % (95 % CI 70–78 %), respectively, after adjusting for confounding factors [3].

Most of the studies reviewed in the ESHRE guideline showed that ovarian reserve status does not predict fecundity over a 6–12-month period. Women with a lower ovarian reserve and regular menstruation appear to have a similar spontaneous pregnancy rate to women of the same age with normal ovarian reserve. However, ovarian reserve testing plays an important role in predicting pregnancy rates in IVF, as it reflects ovarian response during stimulation cycles [3].

Recommendation:

In women with regular menstrual cycles, the panel recommends against using ovarian reserve testing to predict the probability of spontaneous conception within 6–12 months in couples with unexplained infertility.

Grade (strength of recommendation): xxx

Level of evidence: ++

6.2. Ovulation

6.2.1. Should ovulation confirmation tests be done in women with a regular cycle?

A regular menstrual cycle is defined as occurring every 24–38 days and lasting 8 days or less and shortest to longest cycle variation of less than 7–9 days [9]. Several commonly used methods have been described to confirm regular ovulation, including ultrasound, urinary luteinizing hormone (LH) measurement, serum progesterone, basal body temperature, and cervical mucus assessment. Ultrasound monitoring of follicular growth and rupture is considered the gold standard for confirming regular ovulation. Ovulation signs on ultrasound include a decrease in follicle size, corpus luteum formation, pelvic free fluid, and the appearance of a luteinized endometrium. The LH surge begins 35–44 h prior to ovulation, resulting in a urinary LH concentration ranging from 20 to 100 mIU/mL [10].

Urinary LH measurement seems to be as accurate as ultrasound monitoring (97 %), with a sensitivity of 100 % and specificity of 25 %. Two other cohort studies confirmed both the agreement (98–100 %) and accuracy (97 %) between urinary LH testing and ultrasound monitoring. There is a high correlation between quantitative (assay, plasma) and qualitative (colour, urine) LH tests [3].

Ovulation can also be confirmed by a midluteal serum progesterone level of > 3 ng/mL [10]. Progesterone measurement has been estimated to have an accuracy of 79 %, compared to ultrasound. Basal body temperature rise (between 0,2-0,5°C), due to progesterone, has shown a sensitivity of 77 %, a specificity of 33 % and an accuracy of 74 % compared to ultrasound [3]. Cervical mucus changes to a watery, highly receptive liquid, seems to have a 48 % correlation with ultrasound detection of ovulation [11].

There is a lack of evidence regarding the role of above-mentioned ovulation confirmation tests among women with regular menstrual cycles. A prospective study by Chinta P et al. reported a prevalence of anovulatory cycles of 0.5 % and 7.1 % in women with regular cycles, based on ultrasound and mid-luteal serum progesterone assessment, respectively [12]. Pregnancy rates do not appear to be significantly influenced by sporadic anovulatory cycles in women with otherwise regular menstruation [13].

Recommendations:

- In women with regular menstrual cycles, there is no indication of routine ovulation confirmation tests.

Grade (strength of recommendation): GCP

Level of evidence: -

- If ovulation must be confirmed, ultrasound monitoring, urinary LH measurement, mid-luteal serum progesterone can be used.

Grade (strength of recommendation) xxx

Level of evidence: +

6.3. Luteal phase

6.3.1. Testing for luteal phase deficiency in UI

Clinically detected luteal phase defect (LPD) refers to a luteal phase of ≤ 10 days in length, although alternative definitions include ≤ 11 days and ≤ 9 days. It has been shown that 13 % of ovulatory menstrual cycles are associated with luteal phase length of < 10 days and this is not associated with decreased fecundity over 12 months [14].

Multiple diagnostic tests have been proposed, including clinical, biochemical, and histologic tests, but none have been able to reliably differentiate between fertile and infertile women. The measurement of a single or multiple serum progesterone levels and performing an endometrial biopsy are the most proposed methods for diagnosing LPD [14].

A luteal progesterone value of > 3 ng/mL has been described as indicative of ovulation. However, there is no established minimum progesterone value that guarantees normal luteal function. Due to potential variations in progesterone levels resulting from pulsatility and inter-cycle differences, studies suggest that the most reliable marker of luteal phase function is daily progesterone measurements during the luteal phase. A total of three daily progesterone measurements between days 5–9 of the luteal phase, with values < 30 ng/mL, has been proposed as an alternative diagnostic tool for LPD [14].

To date, only one study has investigated mid-luteal progesterone levels in natural conception, reporting the lowest progesterone level associated with pregnancy to be 8.5 ng/mL. However, there are no studies conclusively documenting a minimum mid-luteal serum progesterone level required for pregnancy to occur. Even if a threshold for mid-luteal serum progesterone levels below which pregnancy and live birth rates are decreased is assumed, there is no evidence showing an increase in live birth rates with exogenous progesterone administration in any form [3].

Two studies, one with a large sample size, show that endometrial dating does not effectively differentiate between fertile and infertile women. As a result, there is no need for invasive testing (e.g. endometrial biopsy) in the context of assessing fertility. However, the guideline specifies that this recommendation does not apply to women who need an endometrial biopsy for other medical reasons, such as diagnosing endometrial hyperplasia [3].

Recommendations

- In women with regular menstrual cycles, we do not consider routinely measuring midluteal serum progesterone levels.
- **Grade (strength of recommendation): xxx**

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Level of evidence: ++

– There is no reliable method to diagnose luteal phase deficiency.
Grade (strength of recommendation): xxx
Level of evidence: ++

– In women investigated for infertility, endometrial biopsy for histological examination is not recommended in the absence of other indications.
Grade (strength of recommendation): xxx
Level of evidence: ++

6.4. Tubal factor

6.4.1. Role of Chlamydia IgG testing in predicting tubal patency

Evidence suggests that Chlamydia antibody testing (CAT), used to detect prior Chlamydia infection, may serve as a non-invasive method to distinguish between women at low versus high risk for tubal occlusion. An old systematic review by Mol et al. showed a sensitivity of CAT for tubal pathology of 0,21-0,90 and a specificity between 0,29-1 in patients with subfertility, with the validity of the test depending on the assay used. Thirteen studies that were not included in Mol's review reported a CAT sensitivity of 0.61 (95 % CI 0.54–0.67) and a high specificity of 0.83 (95 % CI 0.78–0.88) with a positive predictive value of 0.58 and negative predictive value of 0.85 [3].

Due to its high specificity, a negative CAT in conjunction with a low-risk medical history may be considered indicative of tubal patency. Given the lower positive predictive value of CAT, both a positive test as well as a negative test with a high-risk medical history should be confirmed with additional tubal patency tests. To emphasize the role for medical history, a study of Hubacher et al. reported tubal pathology laparoscopically in 84,3 % patients with a high-risk history. A medical history of pelvic inflammatory disease (PID), previous genital tract infections, aberrant vaginal discharge and a positive CAT are considered high-risk [3].

Recommendations:

– Chlamydia IgG antibody testing could be used as a non-invasive test to differentiate between women at low versus high risk for tubal occlusion.
Grade (strength of recommendation): xxx
Level of evidence: ++

– In women at high risk of tubal occlusion, based on Chlamydia antibody testing or medical history, tubal patency testing should be performed.
Grade (strength of recommendation): GCP
Level of evidence: -

6.4.2. Which test could be performed to assess tubal patency in UI?

The assessment of tubal patency is a crucial component of the infertility workup, as tubal occlusion, caused by factors such as pelvic inflammatory disease, endometriosis, and, less commonly, polyps or fibroids—accounts for 26 % of female infertility cases [15].

Diagnostic laparoscopy with chromopertubation has historically been the gold standard for assessing tubal patency. However, due to its invasiveness and the requirement for general anesthesia, less invasive and more cost-effective alternatives such as hysterosalpingography (HSG) and hysterosalpingo-contrast/foam sonography (HyCoSy/HyFoSy) are now widely used [15].

In two systematic reviews (SR) and a meta-analysis, together including 44 cohort studies and a total of 3530 patients, the sensitivity and specificity of 2D-HyCoSy for assessing tubal patency was evaluated, using laparoscopy with chromopertubation as the gold standard. For 2D-HyCoSy the pooled sensitivity and specificity were 0.86 (95 % CI 0.80–0.91) and 0.94 (95 % CI 0.90–0.96), respectively. The pooled sensitivity and specificity for 3D- and 4D HyCoSy were 0.92 (95 % CI 0.90–0.94) and 0.92 (95 % CI 0.89–0.93), respectively [4].

HyCoSy, or HyFoSy when foam is used, offers the additional advantage of being performed during the pelvic ultrasound conducted as part of the fertility workup. It allows for simultaneous 3D assessment of the uterine cavity, facilitating the diagnosis of uterine malformations and intra-uterine anomalies such as submucosal fibroids, adhesions, and polyps. Moreover, it is generally better tolerated by patients and more cost-effective than HSG [3, 16].

In a recent systematic review published in 2025, HSG, HyCoSy, and HyFoSy were compared and no significant difference in accuracy was found. However, it was shown that HyFoSy was considered less painful compared to HSG (risk ratio 1.09, 95 % CI 1.00–1.17) [15]. Whether pregnancy rates increase after flushing the fallopian tube with oil-soluble contrast medium following a normal HyCoSy/HyFoSy is still under investigation [17].

In general, both HyCoSy/HyFoSy and HSG can be considered as valid tests to assess tubal patency, particularly if no specific risk factors for tubal disease are present. Given the many advantages of 2D-HyFoSy compared to HSG, it might be a better choice for the initial assessment of tubal patency in women with infertility. Findings suggesting proximal tubal obstruction require further evaluation to exclude transient occlusion resulting from tubal or myometrial contractions [18].

There are no official recommendations regarding routine antibiotic prophylaxis, and in most of the studies included in the SR it was not administered [15]. Although there is no evidence and this is based solely on expert opinion, it can be considered to give all patients Azithromycine 1 g.

Recommendations:

– Hysterosalpingo-foam sonography (HyFoSy) and hysterosalpingography (HSG) are both valid tests for assessing tubal patency when compared to laparoscopy with chromopertubation (methylene blue).
– **Grade (strength of recommendation): xxxx**
– **Level of evidence:** + ++ +
– HSG and HyFoSy are comparable in terms of diagnostic capabilities; however, HyFoSy might be preferred
– **Grade (strength of recommendation): GCP**
Level of evidence: -

6.5. Uterine factor

6.5.1. Which diagnostic procedures should be performed to exclude uterine anomalies in women with unexplained infertility?

In three prospective cohort studies, 2D and 3D ultrasound (US) were compared for the assessment of uterine anomalies. In all three studies, real-time 3D-US showed significantly higher sensitivity and specificity compared to 2D-US. The accuracy of 3D-US was 97.1 %, compared to 51.4 % for initial 2D-US and 82.9 % for expert 2D-US, when compared with hysteroscopy and laparoscopy as the reference standard [3].

Additionally, it was shown that timing the ultrasound evaluation during the second half of the cycle (the luteal phase) is more appropriate for evaluating the uterus for congenital anomalies as secretory endometrium allows better visualization of the contours of the endometrial cavity [19]. Specifically, in the luteal phase, 3D-US demonstrated 100 % sensitivity and 93.7 % specificity, whereas in the follicular phase, sensitivity was 94.7 % and specificity was 75 %. Furthermore, comparing 2D- and 3D-US, the cost and time per scan is the same, and there is no increase in pain or invasiveness for the patient [3]. However, the panel acknowledges that 3D-US may not be available in every clinic and the diagnostic accuracy is performer-dependent.

No relevant studies were identified investigating the use of MRI compared to 2-D-ultrasound to confirm a normal uterine anatomy in women with UI [3].

Recommendation
Ultrasound, preferably 3D-ultrasound, is recommended to exclude uterine anomalies in women with UI.
Grade (strength of recommendation): xxx
Level of evidence: + +

6.5.2. What is the added value of hysteroscopy or HyCoSy in pregnancy outcome of UI?

Hysteroscopy is often applied to exclude intra-cavitary anomalies in infertility patients, irrespective of available guidelines, due to its higher accuracy compared to ultrasound.

There is indeed evidence suggesting that subsequent hysteroscopy or HyCoSy procedures can identify additional uterine anomalies in women with normal findings on 2- or 3D ultrasound. However, most of these anomalies, such as small polyps or a septum, are unlikely to affect pregnancy rates or outcomes, particularly when asymptomatic [20].

In a single randomized study, an increase in the number of cumulative pregnancies was reported after treating findings detected with hysteroscopy. However, due to the low quality of this study the evidence is insufficient to recommend routine hysteroscopy in UI [5]. In contrast, a high-quality multicenter RCT demonstrated that routine hysteroscopy does not provide added value prior to IVF/ICSI. This study included, among others, patients with UI [21]. The reproductive outcomes were also discussed in a Cochrane review, which found no evidence to support the routine use of hysteroscopy [3].

A retrospective cohort study including 294 women explored the effectiveness of routine HyCoSy following normal 2D ultrasound findings, in comparison to targeted HyCoSy. The study group included 124 women with normal ultrasound findings, while the control group consisted of 170 women who had uterine abnormalities identified on 2D ultrasound. Among women with normal ultrasound results, 10.4 % showed uterine abnormalities on HyCoSy, but only 23 % were confirmed via hysteroscopy, and none were verified by pathology [3].

The disadvantages of hysteroscopy and HyCoSy include an increased patient burden and higher costs. The main benefit for the patient might be more certainty regarding the absence of anatomical barriers to implantation. To note is that none of the studies compared 3D-ultrasound as an option and given that some of the included papers were old, the ultrasonography technology may not represent the latest 2D/3D performance [3].

Moreover, as a practical point, given the evidence on the good performance, availability and cost profile of HyCoSy compared to hysteroscopy, these methods should be prioritized in cases where further assessment for uterine cavity is needed.

Recommendation

If ultrasound assessment of the uterine cavity is normal no further evaluation with hysteroscopy, HyCoSy or MRI is needed.
Grade (strength of recommendation): xxxx
Level of evidence: + +

6.6. Laparoscopy

6.6.1. What is the utility of laparoscopy in women with UI?

A study evaluated the accuracy of diagnostic laparoscopy prior to intra-uterine insemination (IUI) and included 495 women with patent tubes on HSG. In 124 women (25 %), the laparoscopy changed the initial treatment plan of IUI. In 21 women (4 %), the abnormalities were severe enough to necessitate a shift to IVF or open surgery. The remaining 103 women (21 %) underwent surgical interventions intended to improve their fertility outcomes [3].

The same group later published a RCT including 154 women comparing a laparoscopy with ablation or resection of stage I/II endometriosis lesions if needed before IUI or six cycles of IUI without a prior laparoscopy. The overall pregnancy rate was not significantly different between the two groups: 44 % (34/77) after immediate laparoscopy and 49 % (38/77) after immediate IUI (OR 1.2, 95 % CI 0.7–2.3), of which 12 vs. 16 spontaneous and 22 vs. 22 IUI pregnancies [3].

A Cochrane review based on moderate quality evidence from 3 RCTs (528 participants) mainly on peritoneal endometriosis found that laparoscopic treatment probably improves viable intrauterine pregnancy rate compared to diagnostic laparoscopy only (odds ratio (OR) 1.89, 95 % CI 1.25–2.86. Data on live birth rates are currently still missing [22, 23].

Diagnostic laparoscopy provides direct visual examination of the pelvic anatomy and is the best method available to diagnose tubal and peritoneal abnormalities, such as pelvic adhesions and endometriotic implants, that may impair fertility [3]. However, the clinical impact of these findings in the presence of tubal patency remains a subject of debate. Given that diagnostic laparoscopy is not without surgical and anesthetic risk and demands a specialized surgical team and operation time, the overall costs may outweigh the potential benefits. Therefore, routine laparoscopy is not recommended for infertile women without a clinical suspicion of pelvic pathology [3].

In cases where pelvic pathology is suspected, such as those with a history of PID, previous ectopic pregnancies or clinically suspected endometriosis a diagnostic laparoscopy can be considered for pelvic assessment and treatment if required [3].

Recommendations:

- Routine laparoscopy is not recommended for the diagnosis of unexplained infertility.

Grade (strength of recommendation): xxxx
Level of evidence: + ++

- Laparoscopy could be considered for women in whom pelvic pathology is suspected or those presenting with symptoms suggestive of endometriosis.

Grade (strength of recommendation): xxx
Level of evidence: + +

6.7. Male factor

6.7.1. Are additional semen tests recommended when semen analysis according to WHO criteria is normal?

At present, semen analysis remains the gold standard for diagnosing male infertility. It assesses sperm quality by evaluating parameters such as sperm count, morphology, and motility. However, the cause of male infertility remains unexplained in up to 50 % of patients using this classic assessment. Therefore, additional diagnostic methods, including the assessment of anti-sperm antibodies and DNA fragmentation, have been explored as tools to identify contributing factors in UI [24].

Table: Lower reference limits (5th centiles and their 95 % Cis) for semen characteristics [24]:

Parameter	2021 Lower reference limit (95 %CI)
Semen volume (mL)	1.4 (1.3–1.5)
Total sperm volume (106/ejaculate)	39 (35–40)
Sperm concentration (106/mL)	16 (15–18)
Total motility (PR+NP,%)	42 (40–43)
Progressive motility(PR,%)	30 (29–31)
Vitality (live spermatozoa, %)	54 (50–56)
Sperm morphology (normal forms, %)	4 (3.9–40)

6.8. Anti-sperm antibodies (ASA)

A case-control study by Bozhedomov et al. demonstrated that ASA are significantly more prevalent in normozoospermic infertile men compared to fertile controls. Specifically, 15.6 % of the infertile men showed a mixed antiglobulin reaction (MAR) $\geq$ 50 %, compared to only 1.9 % in the fertile control group. Additionally, the study showed that in ASA-positive men the acrosome reaction was decreased, DNA fragmentation was higher, and there were increased levels of reactive oxygen species (ROS), each of which are markers of sperm dysfunction that could contribute to the inability to conceive, even in the presence of apparently normal semen parameters [3].

In a cohort study of 84 men the occurrence of natural pregnancies and the effectiveness of IUI were analyzed together with the degree of sperm autoimmunization. In men with 100 % MAR test, natural live birth rate was 2/44 (4.5 %), 14/38 (36.8 %) after IUI and 7/15(46.7 %) after ICSI. In males with moderate (50–99 %) MAR test, the natural live birth rate was 12/40 (30 %), 7/26 (26.9 %) after IUI and 5/6 (83.3 %)

after ICSI. Multiple regression analysis showed that the percentage of MAR test positivity was an independent predictor of natural live birth rate (β −0.06 (95 %CI−0.10 to −0.02) [3]. There are a few older studies on ASA in patients undergoing MAR with most of them showing no difference in pregnancy rates.

Although anti-sperm antibodies may play a role in UI, the current body of evidence is insufficient to recommend routine ASA testing as part of the standard infertility workup. Most studies on ASA in UI are outdated, small, underpowered, or included men with abnormal semen parameters. Furthermore, evidence-based reference values on the appropriate cut-off for positive ASA results is currently lacking. Additionally, to date large, well-conducted studies that demonstrate the effect of anti-sperm antibodies on pregnancy rate or live births are missing [3].

Recommendation

Testing for anti-sperm antibodies (ASA) is not recommended when semen analysis, according to WHO criteria, is normal.

Grade (strength of recommendation): xxx

Level of evidence: + +

6.9. DNA fragmentation test

It is important to note that each sperm DNA fragmentation (SDF) assay currently used in clinical practice is based on a specific technical methodology that detects different structural aspects of sperm DNA damage, which makes them non-interchangeable, as they have often been in the literature [25].

An increased level of sperm DNA fragmentation may adversely affect reproductive success rates in natural and assisted reproduction techniques. This can be explained by impaired embryo development, subsequently leading to a higher miscarriage (17.8 % vs. 39.9 %) and recurrent pregnancy loss rate. Moreover, evidence suggests an increased SDF-rate in infertile men compared to fertile men and a similar rate of SDF in infertile men with normal and abnormal semen parameters. It is suggested that a threshold DNA fragmentation index of 25 % as measured with sperm chromatin structure assay, is associated with reduced pregnancy rates in natural conception or IUI [3, 6, 7].

The clinical relevance of performing SDF tests in couples with UI is questionable, as current evidence on reproductive outcomes is limited to couples undergoing ART. While some think it might help with identifying hidden sperm issues and guide treatment options (lifestyle changes, decreased abstinence, antioxidants, microfluidics sperm sorting, testicular sperm extraction), no studies have directly examined the impact of SDF testing on clinical management or whether fertility outcomes differ between couples who undergo testing and those who do not [6].

Recommendation

DNA fragmentation test is not recommended when semen analysis according to WHO criteria is normal.

Grade (strength of recommendation): xxxx

Level of evidence: + +

6.10. Additional testing

6.10.1. Should thyroid testing be part of the workup in couples with UI?

The impact of thyroid dysfunction on female/male fertility and obstetric/fetal outcome has widely been investigated.

In a small case-control study comparing 44 women with unexplained infertility and 44 fertile women, TSH and T4 seemed to be slightly higher in the women with unexplained infertility [3]. Similar findings were seen in another cross-sectional study comparing thyroid hormones in women with UI versus women in couples with male infertility. TSH was found to be significantly higher in the UI group compared to the couples with male infertility (1.95 (IQR 1.54–2.61 vs. 1.66 (IQR 1.25–2.17) mIU/L). This has also been found in a case control study comparing 300 euthyroid women with UI with women from couples with male infertility (0.62 vs. 0.64 mIU/L, $p < 0.001$). There were more women with unexplained infertility with high-normal TSH values > 2,5 mIU/L, compared to the control group [26].

A retrospective study including euthyroid women undergoing ovulation induction/ intrauterine insemination for UI found that having a preconceptional high-normal TSH value (2,5-4,5 mIU/L) wasn't associated with lower pregnancy rates compared to women with TSH levels between 0,3-2,5 mIU/L [27].

Despite the above-described findings, there is a lack of evidence for treating a high-normal TSH level in women with UI with levothyroxine. A small retrospective study including women with high-normal TSH values and UI compared conception and live birth rate between those who received levothyroxine versus not. Women with UI and high-normal TSH who were treated with levothyroxine seemed to have higher conception rate, but lower live birth rate compared to untreated women [28]. The impact of treating women with unexplained infertility and high-normal TSH with levothyroxine needs further investigation.

Recommendations

- Measuring TSH is considered good practice in preconception care.

Grade (strength of recommendation): GCP

Level of evidence: -

- No additional thyroid evaluation tests are recommended for women if TSH levels are within the normal range
- **Grade (strength of recommendation): xxxx**

Level of evidence: + +

- We do not recommend treating women with high-normal TSH values (TSH 2.5–4.5 mIU/L)

Grade (strength of recommendation): xxx

Level of evidence: + +

7. Quality control

The quality control of this guideline is by an external review. After the approval of all panel members, the preliminary guideline will be made available for 4 weeks to all members of the VVOG through publication on the VVOG website for peer review. The comments that arise during the peer review will be discussed in group with all panel members and adjusted if necessary. The VWRG is responsible for the final approval.

8. Sponsorship and conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

9. Implementation

After approval by the VWRG, this guideline will be published in EJOG and Gunaikeïa, the official journal of the VVOG, as well as on the VVOG website. It will be available to all VVOG members.

10. Suggestions for further scientific research?

Based on the literature review, evidence is insufficient or inconsistent for several described topics. The panel suggests further research on the clinical significance, diagnosis and treatment of LPD in unexplained infertility. There is also a lack of evidence about the impact of levothyroxine treatment for women in couples with unexplained infertility and high-normal TSH values. Further research should also focus on large, well-designed studies to establish evidence-based reference values for ASA testing in UI. Investigating the impact of ASA and SDF on pregnancy rates and live births, particularly in men with normozoospermia, is crucial to determine its clinical relevance in UI and potential role in infertility workup.

11. Limitations

Socio-economic aspects were not considered in this guideline. The opinions and preferences of the target group (couples with UI) were not taken into account when developing this guidance. Finally no cost-benefit analysis was conducted.

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13. Revision

The CG will be revised in 3–5 years by the responsible working group (VWAG / VWOG / VWV / VWRG) unless new high-grade evidence emerges.

Disclaimer

We have taken the utmost care to prepare and maintain this Clinical Guidance paper. Nevertheless, the VVOG accepts no liability for any inaccuracies, nor for damage, adverse effects or inconvenience of any kind (actions, omissions and decisions based on this information).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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