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Unexplained infertility

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Unexplained infertility Guideline Development Group

Developed in collaboration with the Monash University led NHMRC Centre of Research Excellence in Women's Health in Reproductive Life (CREWHIRL).

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Contents

Introduction to the guideline

Approximately 30% of infertile couples are considered to experience "unexplained infertility" (UI) (2019, 2020). This controversial diagnosis is made when no abnormalities of the female and male reproductive systems are identified. UI is inevitably a diagnosis by exclusion, following otherwise "standard" investigations. However, a consensual standardization of the diagnostic work-up is still lacking. The International Committee for Monitoring Assisted Reproductive Technologies (ICMART) defined UI as "infertility in couples with apparently normal ovarian function, fallopian tubes, uterus, cervix and pelvis and with adequate coital frequency; and apparently normal testicular function, genito-urinary anatomy and a normal ejaculate. The potential for this diagnosis is dependent upon the methodologies used and/or those methodologies available" (Zegers-Hochschild et al., 2017). In some instances, the terms "unexplained" and "idiopathic" infertility have been used interchangeably. The definition of idiopathic infertility varies according to previously published reports depending on the hypothesised possible aetiological factors and diagnostic work-up performed by the investigators (Ventimiglia et al., 2021). Generally, idiopathic male infertility is referred in literature to those men from couples where female factor is missing and who display abnormal semen parameters and no known aetiologic factor for their infertility (Krausz, 2011, Schubert et al., 2022). Idiopathic male infertility is considered outside the scope of this guideline.

The proportion of couples with UI is related to the extent of diagnostic examination performed to uncover putative causes for unsuccessful attempts at pregnancy (ESHRE Capri Workshop Group, 2004). Furthermore, the criteria for labelling specific features as "normal" are heterogeneous. Finally, apart from the clearly recognized causes of infertility, several undetectable defects in the reproductive process might prevent conception.

In the absence of an identified cause, the management of UI is traditionally empirical. The efficacy, safety, costs and risks of treatment options have not been subjected to robust evaluation.

Existing guidelines for UI were released from the Canadian Fertility and Andrology Society (Buckett and Sierra, 2019) in 2019 and from the American Society for Reproductive Medicine (ASRM) in 2020 (2020). Both documents exclusively address the treatment of UI.

Based on the lack of comprehensive guidelines, the ESHRE Special Interest Group (SIG) Reproductive Endocrinology initiated the development of an ESHRE guideline focussing on both the diagnosis and the therapeutic management of couples with UI. The guideline was developed according to a well-documented methodology, universal to ESHRE guidelines and described in the Manual for ESHRE guideline development (www.eshre.eu/guidelines). Details on the methodology of the current guideline are outlined in Annex 4.

The guideline development group (GDG) was composed of members of the SIG Reproductive Endocrinology, SIG Andrology, SIG Safety and Quality in ART, and SIG Nursing and Midwifery, and a patient representative from Fertility Europe. This guideline was developed in collaboration with Monash University led Australian NHMRC Centre for Research Excellence in Women's Reproductive Health. The members of the guideline development group are listed in Annex 1.

Guideline scope

Knowledge gaps were identified and prioritized.

The aims of this guideline are:

- To provide clinicians with evidence-based information on the optimal diagnostic work-up for infertile couples based on the examinations and procedures available to date, to correctly establish the diagnosis of UI.
- To provide clinicians with evidence-based information on the optimal therapeutic approach considering issues like live birth rates, safety, patient compliance, and individualization.

This guideline aims at assisting healthcare professionals and couples in decision making about appropriate and effective management of all cases of UI. This could inevitably lead to a certain degree of generalization. Beyond evidence-based recommendations, the GDG acknowledges that each medical decision needs to consider individual characteristics, preferences, beliefs and values. Similarly, even if the guideline is applicable in both high- and low-income settings, all recommendations need to be contextualized, based on different socio-geographical areas, regulations and economic resources.

Even if not specifically and/or comprehensively addressed in this guideline, some aspects of the pre-conception care were included, due to the overlap between the phase of diagnosis/treatment of infertility and the interventions aimed at improving the pregnancy and child-health outcomes.

The guideline consists of four chapters. The first chapter reviews the ICMART definition of UI. The second chapter is about diagnostic tests. Since UI is a diagnosis by exclusion, the GDG first reviewed basic fertility tests. This part is applicable to all patients under investigation for infertility. The GDG also reviewed additional tests to facilitate the diagnosis of UI (section II.9 and II.10). The studied population in these sections is couples with UI specifically. The third chapter covers treatment. The studied population in these sections is couples with UI specifically. In the fourth chapter, the GDG investigated whether there is a difference in quality of life between couples with explained or unexplained infertility.

Target users of the guideline

The target users of this guideline include but are not limited to general practitioners, gynaecologists, andrologists, infertility specialists, and reproductive surgeons.

A patient leaflet is available on the ESHRE website (www.eshre.eu/guideline/UI).

Patient population

The current guideline focusses on couples with UI.

This guideline, in line with the research, terminology and discussion in UI is focused on couples, women and men. The guideline group recognises that there are single women, same sex couples or individuals who are transgender, who do not menstruate, who do not have a uterus or who do not identify with the terms used in the literature. For the purposes of this guideline, we use the terms “couples with unexplained infertility”, “women with unexplained infertility” and “men with unexplained infertility”, however, it is not intended to isolate, exclude, or diminish any individual’s experience nor to discriminate against any group.

TERMINOLOGY AND DEFINITIONS

The current guideline applies the terms and definitions as described in the international glossary on Infertility and Fertility Care (Zegers-Hochschild et al., 2017). Specifically, the term medically assisted reproduction (MAR) refers to reproduction brought about through various interventions, procedures, surgeries and technologies to treat different forms of fertility impairment and infertility. These include ovulation induction, ovarian stimulation, ovulation triggering, all ART procedures, uterine transplantation and intra-uterine, intracervical and intravaginal insemination with semen of husband/partner or donor. A list of further abbreviations can be found in Annex 2.

Outcomes for this guideline

The guideline focuses on outcomes of relevance, accuracy, acceptability, reliability, feasibility, value (in terms of cost-benefit ratio) for the diagnostic tools.

The guideline focuses on outcomes of efficacy, safety and patient-related outcomes for the treatment.

The critical outcomes in this guideline are: live full-term singleton birth, live birth, ongoing pregnancy rate, multiple pregnancies/multiple births.

The important outcomes in this guideline are: clinical symptoms, patient satisfaction, health-related quality of life, cost - effectiveness value.

Other outcomes are: clinical pregnancy rate, adverse pregnancy outcome (including miscarriage, ectopic, stillbirth, preterm delivery), ovarian hyperstimulation syndrome, fetal abnormalities, feasibility, acceptability.

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List of all recommendations

Diagnosis			Level of evidence[1]
Confirmation of ovulation			
1	In women with regular menstrual cycles, tests for confirmation of ovulation are not routinely recommended.	GPP	
2	In women with regular menstrual cycles, if confirmation of ovulation is warranted, tests such as urinary LH measurements, ultrasound monitoring or mid-luteal progesterone measurement can be used.	Conditional	⊕○○○
Oocyte/corpus luteum quality			
3	In women with regular menstrual cycles, it is suggested not to routinely measure midluteal serum progesterone levels.	Conditional	⊕○○○
4	In women investigated for infertility, endometrial biopsy for histological examination is not recommended in the absence of other indications.	Strong	⊕⊕○○
Ovarian reserve			
5	In women with regular menstrual cycles, ovarian reserve testing is not required to identify the aetiology of infertility or to predict the probability of spontaneous conception over 6 to 12 months.	Strong	⊕⊕○○
Tubal factor			
6	Hysterosalpingo-contrast-sonography (HyCoSy) and hysterosalpingography (HSG) are valid tests for tubal patency compared to laparoscopy and chromopertubation.	Strong	⊕⊕⊕○
7	HSG and HyCoSy are comparable in diagnostic capacity, thus selection of the technique depends on the preference of the clinician and the patient.	GPP	
8	Chlamydia antibody testing for tubal patency could be considered a non-invasive test to differentiate between patients at low and at high risk for tubal occlusion.	Conditional	⊕○○○
9	In patients at high-risk for tubal abnormality, visual demonstration of tubal patency is necessary.	GPP	
Uterine factor			
10	Ultrasound, preferably 3D, is recommended to exclude uterine anomalies in women with unexplained infertility.	Strong	⊕○○○
11	MRI is not recommended as a first-line test to confirm a normal uterine structure and anatomy in women with unexplained infertility.	Strong	⊕○○○

[1] The level of evidence reports on the quality of the supporting evidence for each recommendation. More information regarding methodology is available in Annex 4 (page 106). GPP refers to good practice point and is applied for recommendations based primarily on expert opinion.

12	If ultrasound assessment of the uterine cavity is normal, no further evaluation is needed.	Strong	⊕○○○
Laparoscopy			
13	Routine diagnostic laparoscopy is not recommended for the diagnosis of unexplained infertility.	Strong	⊕○○○
Cervical/ vaginal factor			
14	The post-coital test is not recommended in couples with unexplained infertility.	Strong	⊕⊕○○
15	Vaginal microbiota testing could be considered in couples with unexplained infertility only in a research setting.	Research only	
Male genito-urinary anatomy			
16	Testicular imaging is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○
Male additional tests			
17	Testing for anti-sperm antibodies in the semen is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○
18	Testing for sperm DNA fragmentation is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○
19	Sperm chromatin condensation test is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○
20	Sperm aneuploidy screening is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○
21	Serum hormonal testing is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○
22	HPV testing of semen is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○
23	Microbiology testing of semen is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○
Additional tests for systemic conditions			
24	Testing for anti-sperm antibodies in serum of either males or females with unexplained infertility is not recommended.	Strong	⊕○○○
25	Testing for coeliac disease in women with unexplained infertility can be considered.	Conditional	⊕⊕○○
26	Testing for thyroid antibody and other autoimmune conditions (apart from coeliac disease) in women with unexplained infertility is not recommended.	Strong	⊕○○○
27	TSH measurement is considered good practice in pre-conception care.	GPP	
28	No additional thyroid evaluation in women is recommended if TSH is within the normal range.	Strong	⊕○○○

29	Testing for thrombophilia in women with unexplained infertility is not recommended.	Strong	⊕○○○
30	Measurement of oxidative stress in semen of males with unexplained infertility should only be considered in the context of research.	Research only	
31	Measurement of oxidative stress in women with unexplained infertility is not recommended.	Strong	⊕⊕○○
32	Genetic or genomic tests are currently not recommended in couples with unexplained infertility.	Strong	⊕○○○
33	Testing for vitamin D deficiency in women is not recommended for diagnosis of unexplained infertility.	Strong	⊕○○○
34	Prolactin testing in women is not recommended.	Strong	⊕○○○
35	BMI evaluation in women is considered good practice in pre-conception care.	GPP	

Treatment

Expectant management

36	IUI with ovarian stimulation is recommended as a first-line treatment for couples with unexplained infertility.	Strong	⊕○○○
37	The GDG advices to base the decision to start active treatment on prognosis in couples with unexplained infertility.	GPP	

Active treatment

38	IUI with ovarian stimulation is recommended as a first-line treatment for couples with unexplained infertility.	Strong	⊕○○○
39	To avoid multiple pregnancies and OHSS, care is needed by using gonadotrophin treatment only in a low-dose regimen with adequate monitoring.	GPP	
40	IVF is probably not recommended over IUI with ovarian stimulation in couples with unexplained infertility.	Conditional	⊕○○○
41	It is expected that the decision to use IVF is individualized by patient characteristics such as age, duration of infertility, previous treatment and previous pregnancy.	GPP	
42	ICSI is not recommended over conventional IVF in couples with unexplained infertility.	Strong	⊕○○○

Mechanical-surgical procedures

43	Hysteroscopy for the detection and possible correction of intrauterine abnormalities not seen at routine imaging is not recommended.	Strong	⊕⊕○○
44	HSG (i.e., tubal flushing) with an oil-soluble contrast medium is preferable over a water-soluble contrast medium. Risks and benefits of tubal flushing with oil-soluble contrast medium should be discussed with all couples with unexplained infertility.	Conditional	⊕⊕○○
	If incidentally minimal to mild endometriosis is found at laparoscopy, this is not further considered unexplained infertility by the GDG.		Conclusion

45	Endometrial scratching should not be offered for unexplained infertility.	Strong	⊕⊕○○
Alternative therapeutic approaches			
46	Adjunct oral antioxidant therapy to women undergoing fertility treatment is probably not recommended.	Conditional	⊕○○○
47	Adjunct oral antioxidant therapy to males undergoing fertility treatment is probably not recommended.	Conditional	⊕○○○
48	Acupuncture in women is probably not recommended	Conditional	⊕⊕○○
49	Inositol supplementation in women is probably not recommended.	Conditional	⊕○○○
50	Psychological support, including psychotherapy, is recommended for patients when needed.	GPP	
51	A healthy diet and regular exercise, supported by behavioural therapy, when necessary, are recommended.	GPP	
QoL			
52	Healthcare professionals should be aware that - there is probably no difference in QoL between women with unexplained infertility versus women in couples with known causes of infertility, except when the cause of infertility is PCOS, where the QoL is lower. - QoL is probably higher in men from a couple with unexplained infertility compared to men from a couple with known causes of infertility except when the cause of infertility is men with a partner with PCOS, then the men from a couple with unexplained infertility have a lower QoL.	Conditional	⊕○○○

I. Definition

Narrative Question: After how many months of unprotected intercourse should a couple be defined as infertile?

Based on a wide-ranging analysis of 237 studies of unexplained infertility (UI), 85 of these related to the time of unprotected intercourse in their definition of UI; 46.5% specified 1 year, 39.5% specified 2 years and 14%, 3 years.

According to the International Committee for Monitoring Assisted Reproductive Technology (ICMART) definition of infertility, couples should have at least 12 months of regular, unprotected sexual intercourse before fertility interventions may be initiated (Zegers-Hochschild et al., 2017).

Narrative Question: Should frequency of sexual intercourse affect the definition of UI?

The definition of infertility includes a broad reference to "regular" unprotected sex, albeit without specifying what this term entails. In fact, the concept of "regular" coital frequency is extremely variable and particular to each couple, fluctuating not only over time, but also influenced by multiple factors including age, education, race, working status, exercise and mood, amongst others (Gaskins et al., 2018). Hence, applying strict bounds to define regular unprotected sex is not only unfeasible, but also unadvisable and could cause unnecessary stress in those seeking to conceive.

In their seminal study, Wilcox *et al*. assessed prospectively in 221 women whether sexual frequency within the six days preceding ovulation could affect the probability of conception (Wilcox et al., 1995). The authors found that predicted conception rates did not alter significantly when comparing women who perform sexual intercourse daily, every other day or twice during the fertility window. However, the number of conception cycles were indeed lower in those who had intercourse only once within the before mentioned timeframe. These results seem to reiterate that more frequent ejaculations do not seem to be detrimental to sperm quality in men with normozoospermia and, in fact, may even be beneficial (Agarwal et al., 2016). Hence, in couples seeking to conceive, it could be reasonable to advise to increase sexual intercourse to at least every 2-3 days within the fertility window to the extent that such suits their own preference.

The guideline development group (GDG) acknowledges that giving the indication of having sexual intercourse at least every 2-3 days can sometimes be stressful for individuals.

Narrative Question: Should female or male partner's age affect the definition of UI?

Based on the analysis of 237 studies of UI, only 49 related to upper age limits of the female partner. Of these, 12 studies referred to 35 years, 8 studies to age 38 and 16 studies to 40 years old. The rest were fairly well distributed in small numbers between 36, 37, 39 and 42 years.

The ICMART definition of UI only refers to the clinical diagnosis without any reference to the duration of unprotected intercourse or female partner's age (Zegers-Hochschild et al., 2017). This definition could well be further defined for practical purposes by adding 40 years old as the limit of the female partner's age. This was illustrated in a mathematical model, showing that after 2 years of regular unprotected intercourse, the false positive diagnosis of UI is 10% in women under 35 years of age, and increases to 80% in women over 40 years of age (Broer et al., 2011, Somigliana et al., 2016).

To a much lesser extent and at more extreme ages, male age could affect fertility potential (du Fossé et al., 2020, Johnson et al., 2015, Laurentino et al., 2020).

Narrative Question: Should couples with mild infertility factors be included in the definition of UI?

Male

Contextualizing the ICMART definition of UI, GDG considers that the initial fertility evaluation of the male should include at least one basic semen examination (strictly adhering to WHO 6th edition manual for the examination and processing of human semen) from a laboratory subscribed to an external quality control programme, and a reproductive (including sexual history) and medical history. The results of the basic semen examination should be interpreted in conjunction with the findings in clinical examination and history. WHO has developed detailed guidance for history taking (reproductive, sexual, medical) and physical examination of the infertile man (WHO, 2000).

The lower fifth percentile of data from men in the reference population, as described in the WHO manual for the examination and processing of human semen, represents the level under which only results from 5% of the men who achieved conception within 12 months in the reference population were found (WHO, 2021). The GDG proposes that results from a basic semen examination below the lower 5th percentile reference limit (and its 95% confidence interval) should be considered as clinically relevant for decision-making about further clinical investigation. However, anything outside this reference excludes unexplained infertility.

Table 1: The lower fifth percentile of data from men in the reference population (WHO, 2021).

Parameter	5th percentile	95% confidence interval
Semen volume	1.4 ml	1.3-1.5 ml
Sperm concentration	16 x 10^6 per ml	15-18 x 10^6 per ml
Total sperm number	39 x 10^6 per ejaculate	35-40 x 10^6 per ejaculate
Total motility (PR+NP)	42%	40-43%
Progressive motility (PR)	30%	29-31%
Non-progressive motility (NP)	1%	1-1%
Immotile spermatozoa (IM)	20%	19-20%
Vitality	54%	50-56%
Normal forms	4%	3.9-4.0%

Current expert position on the question of repeated basic semen examination is that one single (high quality) ejaculate examination should be sufficient to decide on the following actions of male fertility investigation (Barratt et al. 2017; WHO 2000). However, it is also recommended that semen analysis should be repeated if one or more abnormalities are found (Barratt et al. 2017; WHO 2000).

Going by the ICMART definition of UI which states an apparently normal testicular function and a normal ejaculate (Zegers-Hochschild et al., 2017), mild male factor is excluded from the diagnosis of unexplained infertility.

The GDG acknowledges some studies of unexplained UI have been heterogenous in the inclusion of thresholds for various semen analysis parameters.

Female

Going by the ICMART definition of UI which states an apparently normal ovarian function, fallopian tubes, uterus, cervix and pelvis (Zegers-Hochschild et al., 2017), presence of any female factors excludes the diagnosis of unexplained infertility.

Conclusion

The GDG defines unexplained infertility as follows: infertility in couples with apparently normal ovarian function, fallopian tubes, uterus, cervix and pelvis, age ≤ 40 years and with adequate coital frequency; and apparently normal testicular function, genito-urinary anatomy and a normal ejaculate.

As per the ICMART definition of infertility, couples should have at least 12 months of regular, unprotected sexual intercourse before investigations are initiated.

The GDG recommends routinely taking a medical, reproductive and sexual history from both the male and female partner.

The GDG considers a regular menstrual cycle to be 24 to 38 days, up to 8 days in duration and shortest to longest cycle variation of less than 7 to 9 days (Munro et al., 2018).

The GDG recommends at least one basic semen examination, according to WHO criteria, performed by a laboratory which subscribes to an external quality control programme. If the result from first basic semen analysis is below the lower 5th percentile reference limit as per WHO criteria (6th edition), a second analysis should be performed after a 3-month interval.

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II. Diagnosis

II.1 Confirmation of ovulation

PICO Question: Which is the reliability and convenience of methods to confirm regular ovulation?

Menstrual history

Evidence

No relevant papers were identified that compare menstrual history with other methods to predict/confirm ovulation.

Menstrual history + one progesterone/ ultrasound/ luteinizing hormone urinary measurement

Evidence

In a cohort study, including 101 infertile women with regular menstrual cycles, the accuracy of urinary luteinizing hormone (LH) and serum progesterone tests for the prediction/confirmation of ovulation were determined with ultrasound monitoring as reference standard. The agreement between ultrasound and urinary LH test was 97%. Sensitivity, specificity, and accuracy for LH readings were 100%, 25%, and 97%, respectively. The accuracy of progesterone measurement on day six with ultrasound as reference was 79%, sensitivity and specificity were 80% and 71%, respectively (Guermandi et al., 2001).

Luteinizing hormone (LH) urinary measurement

Evidence

In a cohort study, including 101 infertile women with regular menstrual cycles, the accuracy of urinary LH tests was compared with ultrasound for the prediction/confirmation of ovulation. In 100/101 (97%) cycles, the LH test was in agreement with the ultrasound monitoring, resulting in a sensitivity of 100%, with a specificity of 25% and an accuracy of 97% (Guermandi et al., 2001).

A cohort study, including 32 spontaneously ovulating women, investigated the agreement between quantitative (assay, plasma) and qualitative (colour, urine) LH tests. A high correlation was found between both assays (r=0.688) (Bischof et al., 1991).

A cohort study including 99 spontaneous cycles investigated the agreement between LH urinary test and ultrasound monitoring to predict/confirm ovulation. Positive test results, presumably reflecting the occurrence of a urinary LH surge above 50 IU/L, were observed in 97 (98%) spontaneous cycles (Martinez et al., 1991).

Another cohort study including 55 women with normal ovulatory menstrual cycles investigated the agreement between the LH urinary test and ultrasound monitoring to predict/confirm ovulation. In

39/55 cases (70.91%), ovulation occurred within 24h after positivity of the LH-test (Gregoriou et al., 1990).

SERIAL BASAL BODY TEMPERATURE (BBT)

Evidence

In a cohort study, including 101 infertile women with regular menstrual cycles, the accuracy of basal body temperature (BBT) was compared with ultrasound for the prediction/confirmation of ovulation. In 67/101 cycles, the BBT was in agreement with the ultrasound monitoring, resulting in a sensitivity of 77%, with a specificity of 33% and an accuracy of 74% (Guermandi et al., 2001).

A cohort study including 99 spontaneous cycles investigated the agreement between the thermal nadir and LH test decolouration to predict/confirm ovulation. The BBT nadir correlated with the day of the positive test in 30% of spontaneous cycles (Martinez et al., 1991).

Another cohort study including 55 women with normal ovulatory menstrual cycles investigated the agreement between the thermal nadir and LH test decolouration to predict/confirm ovulation. In 20/55 (36.36%) of the cases, the thermal nadir was noted on the day of decolouration, whereas in 22 (40%) and 13 (23.6%) patients the thermal nadir occurred on days -1 and +1 and on days -2 and days +2 of the LH surge, respectively (Gregoriou et al., 1990).

CHANGES IN THE CHARACTERISTICS OF CERVICAL MUCUS

Evidence

No relevant papers were identified that compare changes in the characteristics of cervical mucus with other methods to predict/confirm ovulation.

ULTRASOUND

Evidence

In the relevant papers identified for this PICO question, follicular growth and rupture monitoring by ultrasound was defined as the gold standard. No relevant papers were identified that investigated the accuracy of ultrasound to predict/confirm ovulation.

OVERALL RECOMMENDATION

Evidence

In the four studies included, follicular growth and rupture monitoring by ultrasound was performed and also defined as the gold standard for this evidence synthesis. In the studies evaluating urinary LH measurements, both the agreement (98-100%) and accuracy (97%) with ultrasound monitoring were very high (Bischof et al., 1991, Gregoriou et al., 1990, Guermandi et al., 2001, Martinez et al., 1991). Meanwhile, BBT and luteal-phase serum progesterone measurements were shown to have estimated accuracies between 70% and 80% (Gregoriou et al., 1990). No studies of sufficient quality in this population could be retrieved to access the predictive value of self-reported menstrual history or changes in cervical mucus to confirm regular ovulation. Moreover, convenience was not formally assessed in any of the studies included.

Recommendation

In women with regular menstrual cycles, tests for confirmation of ovulation are not routinely recommended.	GPP

In women with regular menstrual cycles, if confirmation of ovulation is warranted, tests such as urinary LH measurements, ultrasound monitoring or mid-luteal progesterone measurement can be used.	Conditional	⊕○○○

Justification

Pregnancy would be the most straightforward way to determine if ovulation occurred. However, studies considering pregnancy as reference were not available. Therefore, follicular rupture, evidenced by ultrasound was chosen as the reference test. All included studies presumed that included women had regular cycles which is implicit in the context of unexplained infertility.

In clinical practice, ovulation is seldomly confirmed during basic fertility work-up. If confirmation of ovulation is warranted, all strategies presented a reasonable accuracy to confirm ovulation and may therefore be used. BBT presented with a lower accuracy and was found to be less acceptable to patients. While one may postulate that self-administered testing strategies may be deemed as more convenient for patients, this hypothesis is yet to be adequately tested in the infertile population. Regardless, it is also important to note that the documentation of an ovulation episode in one specific menstrual cycle is not a surrogate marker of regular ovulation.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.1).

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II.2 Oocyte/corpus luteum quality

PICO QUESTION: WHAT IS THE RELIABILITY OF PARAMETERS DETECTING GOOD OOCYTE/ CORPUS LUTEUM QUALITY?

MID-LUTEAL PHASE PROGESTERONE LEVELS

Evidence

A cohort study, including 138 cycles from 72 women with no physical cause for infertility investigated the association between midluteal serum progesterone and conception and reported that the lowest progesterone threshold for conception cycles was 8.5 ng/ml (equals 27.03 nmol/L) (Hull et al., 1982).

Recommendation

In women with regular menstrual cycles, it is suggested not to routinely measure midluteal serum progesterone levels.	Conditional	⊕OOO

Justification

There was only one study, which identified midluteal progesterone levels in natural conception and reported the lowest progesterone level to obtain pregnancy. There are no studies conclusively documenting a minimum midluteal serum progesterone level required for the occurrence of pregnancy. Even if the presence of a threshold of midluteal serum progesterone level 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.

On the other hand, recent data from studies on frozen embryo transfer in a natural cycle and one study involving women with UI undergoing ovarian stimulation (OS) and intrauterine insemination (IUI) suggest an association between luteal phase progesterone levels and probability of a pregnancy and live birth (Gaggiotti-Marre et al., 2020, Hansen et al., 2018). Given the limited information on an association between luteal progesterone levels and spontaneous pregnancy this is an area that requires further research.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.2).

ENDOMETRIAL BIOPSY

Evidence

In an RCT, 287 ovulatory female partners of infertile couples and 332 fertile controls were randomized to undergo histological examination of an endometrial biopsy in the midluteal or late luteal phase of the menstrual cycle. The prevalence of out of phase endometrial biopsy results were similar between fertile and infertile women in adjusted analyses. Receiver operating characteristics (ROC) curves showed less than 0.5 area under the curve (AUC) values for endometrial biopsy to differentiate fertile and infertile women (Coutifaris et al., 2019).

In a cohort study, including 20 women with UI and 21 fertile controls, midluteal endometrial biopsies were performed. Women in the UI group showed similar endometrial maturation as the fertile control group (Edi-Osagie et al., 2004).

Recommendation

In women investigated for infertility, endometrial biopsy for histological examination is not recommended in the absence of other indications.	Strong	⊕⊕OO

Justification

Two studies, one with a large sample size, show that endometrial dating does not discriminate between fertile and infertile women. There is no justification for an invasive test in the context.

This recommendation does not apply to women having an indication for endometrial biopsy, such as endometrial hyperplasia.

The GDG is aware of other methods to assess the endometrium, however, these were not investigated.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.2).

FERTILIZATION FAILURE

Evidence

No relevant papers could be identified investigating the reliability of fertilization failure to determine good oocyte or corpus luteum quality.

EUPLOID EMBRYO RATE WITH PGT-A

Evidence

No relevant papers could be identified investigating the reliability of euploid embryo rate (determined by PGT-A) to determine good oocyte or corpus luteum quality.

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II.3 Ovarian reserve

PICO Question: Should one or more tests of ovarian reserve be included in the diagnostic work-up?

Anti-Müllerian hormone (AMH)

Evidence

In a cohort study, female partners of 148 couples with unexplained infertility (UI) and females from 112 couples with male factor infertility were prospectively compared. Women with serum FSH levels >10 IU/L were excluded. While a multivariate analysis adjusted for age suggested lower anti-Müllerian hormone (AMH) levels being significantly associated with UI, antral follicle count (AFC) was not found to be associated with UI (Yücel et al., 2018).

Women between 30 and 44 years of age, who were trying to conceive for less than three months or were about to start trying to conceive, were prospectively observed in a cohort study. Analyses adjusted for age, body mass index, race, current smoking status, and recent hormonal contraceptive use, showed that women with low AMH values (<0.7 ng/mL, n = 84) had a similar predicted probability of conceiving by six cycles of attempt (65%, 95% CI 50-75%) compared with women (n = 579) with normal values (62%, 95% CI 57-66%) or by 12 cycles of attempt (84% (95% CI 70-91%) vs. 75% (95% CI 70-79%), respectively). Likewise, women with high serum FSH values (>10 mIU/mL, n = 83) had similar predicted probability of conceiving after six cycles of attempt (63%, 95% CI 50-73%) compared with women (n = 654) with normal values (62%, 95% CI 57-66%) or after 12 cycles of attempt (82% (95% CI 70-89%) vs. 75% (95% CI 70-78%), respectively). The study excluded women <30 years of age. Male partners not having provided a semen sample can be considered as a limitation if women with low ovarian reserve would be more likely to have partners with impaired semen. While all women were not enrolled in their first three cycles of attempt; findings were similar when less than 10% of women who entered after their third cycle of attempt were excluded (Steiner et al., 2017).

In another cohort study, 102 women, aged 18 to 46 years, were prospectively followed for 12 cycles. Analyses adjusted for age showed no predictive value of AMH, basal FSH or the AFC for time to ongoing pregnancy (hazard ratio (HR) 1.43, 95% CI 0.84-2.46; HR 0.96, 95% CI 0.86-1.06 and HR 1.03, 95% CI 1.00-1.07, respectively) (Depmann et al., 2017).

Similar AMH levels and AFC were reported in 382 female partners of infertile couples and 350 women with no history of infertility. Moreover, the proportion of women with very low serum AMH levels (with two different cut-offs of serum AMH <5 pmol/L or AFC <7) was similar in two cohorts. The findings were similar when the analyses were restricted to women with UI. All analyses were adjusted for age and other relevant factors (Hvidman et al., 2016).

In a small cohort study, 83 women with UI were prospectively followed for 6 cycles. Serum AMH and FSH levels, as well as AFC were similar between 14 women who achieved a spontaneous pregnancy during the observation period and 69 women who did not. AUC values for AMH, FSH and AFC for prediction of a spontaneous pregnancy were 0.39 (95% CI 0.25-0.52), 0.42 (95% CI 0.25-0.58) and 0.42 (95% CI 0.26-0.57), respectively. Moreover, pregnancy and live birth rates were similar between women with AMH levels <0.75 ng/mL and above (Casadei et al., 2013).

In a small cohort study, cycle day 2 AMH levels were compared between 42 women with UI and 29 women with male factor infertility. Median serum AMH levels were similar between UI and male factor groups (19.3 pmol/L (range 1.3-60.8 pmol/L; equals 2.7 ng/ml, range 0.18-8.5) vs. 21.1 pmol/L (range 5.3-60.8, equals 2.95 ng/ml, range 0.74-8.5 respectively)). AMH alone was a poor predictor of live birth in five years (Murto et al., 2013).

In a cohort study, 186 couples who attempted pregnancy for six menstrual cycles were prospectively observed. Women were between 20 and 35 years old. Compared with women in medium serum AMH levels (quintiles 2 – 4 of the study population), women with low AMH levels (lowest quintile) had similar fecundability (HR 0.81, 95% CI 0.44-1.40), while women with high AMH (in quintile 5) had lower fecundability (HR 0.62, 95% CI 0.39-0.99). Analyses were adjusted for woman's age, BMI, smoking, diseases affecting fecundability, and oligozoospermia. When women with irregular cycles were excluded the high AMH group still had lower fecundability rate (FR, i.e., monthly probability of conceiving) (FR 0.48, 95% CI 0.27–0.85) (Hagen et al., 2012).

In a cross-sectional study, AMH, FSH and AFC were compared between 227 women with strictly defined UI and 226 control women. Women were aged between 25 and 40 years and required to have a serum FSH level <12 IU/L on cycle day 3 within the previous year. Analyses adjusted for age, race, BMI, smoking status and recruitment site showed similar AMH levels and AFC in the two groups. It should be noted that inclusion of women with FSH >12 IU/L during the previous year, might have yielded different results (Greenwood et al., 2017).

In a retrospective study including 325 couples who presented for assessment of infertility and did not have an absolute indication for IVF/ICSI (e.g., bilateral tubal blockage), serum AMH level was not a significant predictor of natural conception in a Cox regression analysis adjusted for female age, type of infertility (primary or secondary), duration of infertility, and percentage of motile sperm (Nguyen et al. 2022). However, the addition of AMH level into the Hunault model was found the improve the accuracy of the model to some extent (Hunault et al., 2004, Nguyen et al., 2022).

Antral follicle count (AFC)

Evidence

A cohort study, including 83 women with UI undergoing six months of expectant management, reported a spontaneous pregnancy rate of 17% (14/83). Antral follicle count (AFC) was not predictive of spontaneous pregnancy with an AUC of 0.418 ± 0.08 (95% CI 0.26-0.57) (Casadei et al., 2013).

In another cohort study, 102 women, aged 18 to 46 years, were prospectively followed for 12 cycles. Analyses adjusted for age showed no predictive effect of AMH, basal FSH or the AFC for time to ongoing pregnancy (HR 1.43, 95% CI 0.84-2.46; HR 0.96, 95% CI 0.86-1.06 and HR 1.03, 95% CI 1.00-1.07, respectively) (Depmann et al., 2017).

Similar AMH levels and AFC were reported in 382 female partners of infertile couples and 350 women with no history of infertility. Moreover, proportion of women with very low serum AMH levels (with two different cut-offs of serum AMH <5 pmol/L or AFC <7) was similar in two cohorts. The findings were similar when the analyses were restricted to women with UI. All analyses were adjusted for age and other relevant factors (Hvidman et al., 2016).

A cohort study compared cycle day 2-4 AFC in 148 women with UI and 112 women with male factor infertility. Women with UI had lower AFC than the male factor group (9 (3-16) vs. 10 (3-23) respectively). Log regression with infertility as the dependent showed that AFC was not significantly associated with UI, after adjusting for age (Yücel et al., 2018).

Rosen *et al.* compared 881 women with UI with 771 women with regular ovulatory cycles from a community study. Women aged between 25 and 40 years in the UI group had significantly lower AFC than similarly aged women in the community group, women between 40 and 45 years had similar AFC in both groups. Serum FSH levels were significantly higher in UI women who were 31–35 years of age and show a tendency to be higher in UI women who were 25–30 years of age. There were no differences in FSH concentrations between groups in women who were 36–40 or 41–45 years of age. However, the authors employed bivariate comparisons for 5-year age brackets rather than using a multivariate model adjusting for significantly lower age in the community group (Rosen et al., 2011).

In a cross-sectional study, AMH, FSH and AFC were compared between 227 women with strictly defined UI and 226 control women. Women were aged between 25 and 40 years and required to have a serum FSH level <12 IU/L on cycle day 3 within the previous year. Analyses adjusted for age, race, BMI, smoking status and recruitment site showed similar AMH levels and AFC in the two groups. It should be noted that inclusion of women with FSH >12 IU/L during the previous year, might have yielded different results (Greenwood et al., 2017).

Day 3 FSH and estradiol

Evidence

A cohort study, including 750 women without infertility, found no difference in cumulative probability of conception for women with FSH>10 IU/L (HR 1.22, 0.92-1.62) after adjusting for confounding factors (Steiner et al., 2017).

A cohort study compared cycle day 2-4 FSH in 148 women with UI and 112 women with male factor infertility. Women with UI had similar FSH compared to the male factor group (7.52 (range 4.21-9.88) IU/L vs. 6.96 (range 5.1-9.37) IU/L respectively). Likewise, estradiol levels were similar in UI and male factor (51.5 (range 27-86) pg/ml vs. 43.5 (range 25-71) pg/ml) (Yücel et al., 2018).

Clomiphene Citrate Challenge Test (CCCT)

Evidence

A cohort study including 236 women from the general ovulating infertility population found that 52% of women with UI (12/32) had an abnormal Clomiphene citrate challenge test (CCCT) as compared to 17.4% for oligo/anovulation, 8.7% for male factor, 4.3% for tubal factor, 4.3% for endometriosis, and 0% for pelvic adhesions. Women with an abnormal CCCT were less likely to conceive as compared to women with a normal result (Scott et al., 1993).

Ovarian volume, ovarian blood flow, inhibin B

Evidence

A cohort study, including 750 women without infertility, found no association of inhibin B levels and cumulative probability of conception (HR 0.999, 0.997-1.001, per 1 pg/ml increase in inhibin B level) after adjusting for confounding factors (age, body mass index, race, current smoking status, and recent hormonal contraceptive use) (Steiner et al., 2017).

A cohort study compared cycle day 2-4 ovarian volume in 148 women with UI and 112 women with male factor infertility. Women with UI had similar ovarian volume as compared to the male factor group (6.2 ml (range 3.2-10.96) vs. 6.06 ml (range 3.3-12.2) respectively). Likewise inhibin B levels were similar in UI and male factor (119 pg/ml (range 40-145) vs. 120 pg/ml (range 52-150)) (Yücel et al., 2018).

In a small cohort study cycle day 2-5 inhibin B levels were compared between 42 women with UI and 29 women with male factor infertility. Median serum inhibin B levels were similar between UI and male factor groups (37.1 (range 7.0-95.4) vs. 47.5 (range 13-138.4) pg/ml respectively). Inhibin B alone was a poor predictor of live birth in 5 years (Murto et al., 2013).

Overall recommendation

Recommendation

In women with regular menstrual cycles, ovarian reserve testing is not required to identify the aetiology of infertility or to predict the probability of spontaneous conception over 6 to 12 months.	Strong	⊕⊕OO

Justification

The term "ovarian reserve" often refers to the quantity of primordial follicles in a woman's ovaries at a given time. Although the term has also been used in a broader sense to include quality of oocytes, it is difficult to assess oocyte quality during the diagnostic work up.

The purpose of diagnostic work up is to identify any factor preventing pregnancy or decreasing spontaneous fecundity and to inform management strategy.

For the first aim, ovarian reserve would be relevant if regularly ovulating women in different categories of ovarian reserve, i.e., low, normal or high, have different conception rates when every other factor is similar, chronological age in particular. Since women cannot be randomized to different categories of ovarian reserve, this question can be answered by two different study designs: comparing conception rates between women with different ovarian reserve (prospective cohort study or cross-sectional study) or comparing ovarian reserve status between fertile women and women with UI (case-control study).

Most of the listed studies consistently show that ovarian reserve status is not predictive of spontaneous conception over the subsequent 6-12 months. As long as they maintain regular menstrual cycles, women with decreased ovarian reserve seem to have similar spontaneous pregnancy rate with women of similar age who have normal ovarian reserve. These observations effectively exclude decreased ovarian reserve per se as a reason for infertility. Thus, an ovarian reserve test (ORT) is not required from a diagnostic standpoint.

ORT would be relevant for choice of management if ovarian reserve status is a determinant of the probability of pregnancy with expectant management, OS-IUI or IVF. Studies reviewed above do not suggest that ovarian reserve status would determine the probability of a spontaneous pregnancy in 6-12 months, so ORT may not be informative to predict the success of expectant management over such a period. While one retrospective study suggests improved accuracy of the Hunault model used to categorize couples based on their anticipated chance of spontaneous conception, it needs to be validated prospectively and for other settings where referral by primary care is not required before consulting a fertility specialist (Hunault et al., 2004, Nguyen et al., 2022). While a retrospective study including 3019 women younger than 35 years old, who underwent IUI in a natural or stimulated cycle, reported similar cumulative live birth rates up to seven IUI cycles between women with serum AMH levels less than 1 ng/ml and higher than 1 ng/ml (Tiegs et al., 2020), another retrospective study including 1861 gonadotropin stimulated IUI cycles without an age limit reported that women in the lower 25th percentile of the study population for serum AMH levels, or women with serum AMH level <0.7 ng/ml were significantly less likely to achieve a clinical pregnancy over six cycles as compared with women higher AMH levels (Vagios et al., 2021). Another retrospective study including 195 couples also reported a positive correlation between serum AMH level and cumulative pregnancy rate over three OS-IUI cycles (Bakas et al., 2015). It should be noted that the study populations were not limited to UI in the latter two studies and a variety of OS protocols have been used. In addition to the limitations of retrospective design, these features introduce some heterogeneity and limit generalizability of findings to UI. It is uncertain whether ovarian reserve status determines the probability of pregnancy with OS-IUI cycles, where the aim should be to limit the number of growing follicles to 2 – 3 to prevent multiple pregnancies. It may be inappropriate to exclude women from OS-IUI based on ovarian reserve status.

On the other hand, ovarian response, hence ovarian reserve, is a major determinant of cumulative probability of live birth per OS cycle in IVF. Women with decreased ovarian reserve will have lower pregnancy/live birth rate per cycle with IVF (because of a low number of oocytes/stimulation) compared to women with similar characteristics but higher ovarian reserve. Thus, diverting women with low ovarian reserve directly or rapidly to IVF is questionable.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.3).

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II.4 Tubal factor

PICO QUESTION: WHAT IS THE ACCURACY OF COMMONLY USED TESTS OF TUBAL PATENCY?

HYSTEROSALPINGO-CONTRAST-SONOGRAPHY (HYCOSY/HYFOSY) VS. LAPAROSCOPY AND CHROMOPERTUBATION TEST

Evidence

A systematic review and meta-analysis including 1977 patients with subfertility in 21 studies investigated the sensitivity and specificity of hysterosalpingo-contrast-sonography (HyCoSy/HyFoSy) in the diagnosis of tubal pathology using laparoscopy with chromopertubation as the reference standard (Alcázar et al., 2020). 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 likelihood ratio (LR) for detecting tubal occlusion with 2D-HyCoSy were 0.14 (95% CI 0.08–0.23) for LR+ and 0.14 (95% CI 0.1–0.2) for LR-, respectively. High heterogeneity was found for sensitivity ($p<0.001$) and for specificity ($p<0.001$). For 3D/4D-HyCoSy the pooled sensitivity and specificity were 0.95 (95% CI 0.89–0.98) and 0.89 (95% CI 0.82–0.94), respectively. The LR for detecting tubal occlusion with 3D/4D-HyCoSy were 0.09 (95% CI 0.05–0.16) for LR+, and 0.06 (95% CI 0.03–0.13) for LR-, respectively. Both sonography methods had almost identical areas under the curve (0.96 for 2D-HyCoSy and 0.97 for 3D/4D-HyCoSy) (Alcázar et al., 2020).

Another systematic review and meta-analysis including 1553 patients in 23 studies investigated the sensitivity and specificity of 3D- and 4D-HyCoSy for tubal patency using laparoscopy as the gold standard. The pooled estimates of sensitivity and specificity were 0.92 (95% CI 0.90–0.94) and 0.92 (95% CI 0.89–0.93), respectively. The area under the ROC curve was 0.97 (95% CI 0.95–0.98) (Wang and Qian, 2016).

Table 1 includes all other studies included as for evidence on the comparison between HyCoSy and laparoscopy and chromopertubation that were not included in the meta-analyses. True and false positive and true and false negative data were extracted from the included publications, followed by calculations on sensitivity, specificity, predictive values and likelihood ratios by the GDG. The unadjusted pooled accuracy of HyCoSy showed a sensitivity of 0.87 (95% CI 0.74–1.00) and a specificity of 0.83 (95% CI 0.77–0.90) (Table 1).

Table 1: Accuracy of HyCoSy compared to gold standard laparoscopy and chromopertubation for tubal patency testing. True and false positive and true and false negative data were extracted from the included publications followed by calculations on sensitivity, specificity, predictive values and likelihood ratios by the GDG. PPV: positive predictive value, NPV: negative predictive value, LR: likelihood ratio.

Reference	Method	No of patients	Clinical background	True positive	False positive	True negative	False negative	Sensitivity	Specificity	PPV	NPV	LR+	LR-
(Chen et al., 2019)	4D-HyCoSy contrast medium	34	Not specified	23	4	32	3	0.88	0.89	0.85	0.91	7.96	0.13
(Cimen et al., 1999)	HyCoSy contrast medium	47	No patients included with a suspicion of acute or chronic PID	9	3	22	2	0.82	0.88	0.75	0.92	6.82	0.21
(Liang et al., 2019)	3D-HyCoSy contrast medium	83	Not specified	86	8	58	10	0.90	0.88	0.91	0.85	7.39	0.12
(Malek-Mellouli et al., 2013)	HyCoSy saline	40	No vaginal, cervical, or pelvic infection	21	8	44	7	0.75	0.85	0.72	0.86	4.88	0.30
(Radić et al., 2005)	saline	37	No patients with any signs of pelvic infection	47	30	58	0	1.00	0.66	0.61	1.00	2.93	0.00
	contrast medium			47	20	68	0	1.00	0.77	0.70	1.00	4.40	0.00
(Rezk and Shawky, 2015)	HyCoSy saline	104	56.7% of patients with history of pelvic surgery	15	4	75	10	0.60	0.95	0.79	0.88	11.85	0.42
(Shahid et al., 2005)	HyCoSy contrast medium	15	History suggestive of ovulatory factors (PCO), pelvic inflammatory disease and endometriosis was noted.	7	0	7	1	0.88	1.00	1.00	0.88	N/A	0.13
		19		5	0	2	12	0.29	1.00	1.00	0.14	N/A	0.71
(Zhou et al., 2012)	3D-HyCoSy contrast medium	75	No acute or subacute inflammation of the reproductive system	72	10	63	5	0.94	0.86	0.88	0.93	6.83	0.08

HYSTEROSALPINGOGRAPHY (HSG) VS. LAPAROSCOPY AND CHROMOPERTUBATION TEST

Evidence

A systematic review and meta-analysis including seven studies with 4521 women investigated the sensitivity and specificity of hysterosalpingography (HSG) in the diagnosis of tubal pathology using laparoscopy with chromopertubation as the reference standard (Broeze et al., 2011). The sensitivity of HSG for any tubal pathology ranged between 46% and 100% and specificity between 73% and 100% across the studies. The unadjusted pooled accuracy of HSG showed a sensitivity of 0.70 (95% CI 0.66–0.74) and a specificity of 0.78 (95% CI 0.75–0.80). After imputation of missing laparoscopy results (for 2632 women), these rates were 0.53 (95% CI 0.50–0.57) and 0.87 (95% CI 0.86–0.88) for sensitivity and specificity, respectively. In women with a low-risk clinical history (no previous pelvic inflammatory disease (PID) and with negative chlamydia antibody testing result), the sensitivity of HSG for detecting unilateral tubal pathology was 38% versus 61% in women with a high-risk history (previous PID and with negative chlamydia antibody testing result). For bilateral tubal pathology, sensitivity ranged between 0% and 100% and specificity ranged between 87% and 97% across the individual studies. The pooled estimates for sensitivity and specificity were 0.66 (95% CI 0.55–0.75) and 0.91 (95% CI 0.89–0.93), respectively. After imputation of laparoscopy results, these rates were 0.46 (95% CI 0.41–0.51) and 0.95 (95% CI 0.94–0.95) (Broeze et al., 2011).

Table 2 includes 18 studies from the evidence review by the GDG that were not included in the systematic review and meta-analysis by Broeze *et al.* Only studies were included that had data available to calculate test performance by the GDG. True and false positive and true and false negative data were extracted from the included publications followed by calculations on sensitivity, specificity, predictive values and likelihood ratios. To support the systematic review by Broeze *et al.* showing high sensitivity and specificity for HSG, the pooled sensitivity and specificity of these additional 18 studies were 0.86 (95% CI 0.78–0.94) and 0.79 (95% CI 0.72–0.86), respectively.

Table 2: Accuracy of HSG compared to gold standard laparoscopy and chromopertubation for tubal patency testing. True and false positive and true and false negative data were extracted from the included publications followed by calculations on sensitivity, specificity, predictive values and likelihood ratios by the GDG. PPV: positive predictive value, NPV: negative predictive value, LR: likelihood ratio.

Reference		No of patients	Clinical background	True positive	False positive	True negative	False negative	Sensitivity	Specificity	PPV	NPV	LR+	LR-
(Adelusi et al., 1995)		104	Not specified	42	21	33	8	0.84	0.61	0.67	0.80	2.16	0.26
(Agrawal and Fayyaz, 2019)		103	No active genitourinary infection	38	31	34	0	1.00	0.52	0.55	1.00	2.10	0.00
(Berker et al., 2015)	Bilateral	264	Not specified	25	9	175	1	0.96	0.95	0.74	0.99	19.66	0.04
	Unilateral			13	30	175	2	0.87	0.85	0.30	0.99	5.92	0.16
(Chang et al., 1987)		1267	Not specified	944	95	171	57	0.94	0.64	0.91	0.75	2.64	0.09
(Dabekausen et al., 1994)		34	Not specified	7	5	17	5	0.58	0.77	0.58	0.77	2.57	0.54
(Foroozanfard and Sadat, 2013)	All	62	No prior pelvic surgery, no history of pelvic infection	9	10	35	8	0.53	0.78	0.47	0.81	2.38	0.61
	Bilateral			6	3	35	1	0.86	0.92	0.67	0.97	10.86	0.16
(Gündüz et al., 2021)		208	No chronic disease or history of abdominal surgery	61	47	86	14	0.81	0.65	0.56	0.86	2.30	0.29
(Hamed et al., 2009)		88	No pelvic infections or organic lesions	36	16	54	8	0.82	0.77	0.69	0.87	3.58	0.24
(Hiroi et al., 2007)	Bilateral	314	Patients without background factor	18	15	192	12	0.60	0.93	0.55	0.94	8.28	0.43
	Unilateral			15	11	192	39	0.28	0.95	0.58	0.83	5.13	0.76
(Ismajovich et al., 1986)		215	Not specified	53	34	88	40	0.57	0.72	0.61	0.69	2.04	0.60
(Keltz et al., 2006)		210	9.04% of patients reported a prior history of Chlamydia infection or PID	40	3	19	11	0.78	0.86	0.93	0.63	5.75	0.25
(Loy et al., 1989)		77	Not specified	16	16	41	4	0.80	0.72	0.50	0.91	2.85	0.28
(Ngowa et al., 2015)	Bilateral	208	Not specified	25	3	27	24	0.51	0.90	0.89	0.53	5.10	0.54
	Distal			59	26	19	9	0.87	0.42	0.69	0.68	1.50	0.31

Reference		No of patients	Clinical background	True positive	False positive	True negative	False negative	Sensitivity	Specificity	PPV	NPV	LR+	LR-
(Rezk and Shawky, 2015)		104	56.7% of patients with history of pelvic surgery	11	3	75	15	0.42	0.96	0.79	0.83	11.00	0.60
(Rice et al., 1986)		143	Not specified	58	11	62	12	0.83	0.85	0.84	0.84	5.50	0.20
(Tan et al., 2021)		644	20.97% (n = 181) of patients had a history of previous pelvic surgery.	477	3	143	21	0.96	0.98	0.99	0.87	46.61	0.04
(Tshabu-Aguemon et al., 2014)		96	patients investigated for tubal infertility	45	11	20	20	0.69	0.65	0.80	0.50	1.95	0.48
(Tvarijonaviciene and Nadisauskiene, 2008)		149	No previous laparoscopic or abdominal tubal surgery related to infertility.	48	47	43	11	0.81	0.48	0.51	0.80	1.56	0.39

Recommendation

Hysterosalpingo-contrast-sonography (HyCoSy) and hysterosalpingography (HSG) are valid tests for tubal patency compared to laparoscopy and chromopertubation.	Strong	⊕⊕⊕○

HSG and HyCoSy are comparable in diagnostic capacity, thus selection of the technique depends on the preference of the clinician and the patient.	GPP

Justification

High risk for tubal occlusion includes past chlamydia infection, PID, peritonitis, known endometriosis and/or pelvic surgery including salpingectomy for ectopic pregnancy. As for the evidence, not all studies described whether the test was done in low/high-risk population. In most of the cases the population was selected to be of low-risk, and this has to be taken into consideration in the recommendation formulated by the GDG. The mechanical tubal flushing has been considered as “treatment” and has been evaluated for evidence in section III.3 mechanical-surgical procedures.

HyCoSy

The current evidence for HyCoSy compared to laparoscopy and chromopertubation, consisting of two systematic reviews and meta-analyses including 44 cohort studies and eight additional cohort studies not included in the meta-analysis, showed that HyCoSy is a valid test for tubal patency. Even though there was high variation in data, there was overall high specificity and sensitivity for HyCoSy. All sonography types (2/3/4D) performed well.

The GDG cannot formulate a recommendation on the use of contrast medium, foam or saline due to too little studies. It has to be noted that the evidence synthesis included studies including contrast medias that are off-label use and some are no longer at the market. A recent systematic review and meta-analysis investigated the frequency of severe pain perception during HyCoSy with different contrast agents (contrast media, saline or foam) and found similar occurrence of mild, moderate and severe pain for all types of contrast during the procedure (Boned-López et al., 2021).

HSG

The current evidence, consisting of a systematic review and meta-analysis including seven cohort studies and 14 additional cohort studies not included in the meta-analysis, showed that HSG is a valid test for tubal patency and less costly and harmful than laparoscopy. The risk for HSG (oil or water-based contrast media) has been evaluated to be low in a recent study by Roest *et al*. including 3289 HSG cases; overall complication risk was 5.1% for oil-based HSG and 1.8% for water based HSG (Roest et al., 2020). The same study also reported intravasation in 4.8% of cases for oil-based contract and 1.3% for the water-based (Roest et al., 2020). Procedure-related PID was rare (0.3% for oil-based contracts and 0.4% for water-based) and no pulmonary embolism or deaths were reported. Clinical history increases the accuracy of HSG testing although as a limitation, HSG has very limited possibility to detect abdominal adhesions compared to laparoscopy.

HyCoSy (using saline or foam) is less harmful than laparoscopy or HSG, given that the women going through HyCoSy can be assessed immediately after ultrasound, allowing the evaluation of the fallopian tubes and uterine cavity in one test. Furthermore, there is no need for general anaesthesia or exposure to radiation with the use of HyCoSy/HyFoSy.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.4).

Chlamydia antibody testing vs. laparoscopy and chromopertubation test

Antibodies against Chlamyida Trachomatis (CT), can be maintained in sera for at least 10 years after infection (Horner et al., 2013, Horner et al., 2016) and are the only available means for determining prior CT infection.

Evidence

A systematic review and meta-analysis including 2729 patients with subfertility in 23 studies investigated the sensitivity and specificity of Chlamydia antibody titres in the diagnosis of tubal pathology using laparoscopy with chromopertubation as the reference standard (Mol et al., 1997). The sensitivity of Chlamydia antibody testing (CAT) for tubal pathology varied between 0.21 and 0.90, with the specificity varying between 0.29 and 1. There was substantial heterogeneity between studies also with regards to the method used for verifying tubal pathology. The discriminative capacity of CAT was significantly different between studies using microimmunofluorescence (MIF) or immunofluorescence (IF) and ELISA or immunoperoxidase (IP) with MIF/IF and ELISA performing equally and IP showing the lowest performance in the estimated summary ROC curve (Mol et al., 1997).

The 13 studies that were not included in the systematic review and meta-analysis by Mol *et al*. (Table 3), showed similar results, pooled sensitivity was quite low 0.61 (95% CI 0.54-0.67), but specificity was as high as 0.83 (95% CI 0.78-0.88). This also reflected the positive and negative predictive value for the antibody testing (pooled PPV 0.58 and NPV 0.85). Combining medical history or transvaginal ultrasound (TVUS) with CAT increased the test performance (Akande et al., 2003, Coppus et al., 2007, Logan et al., 2003). Moreover, quantitative titer threshold could also reveal severity of damage (Akande et al., 2003).

Recommendation

Chlamydia antibody testing for tubal patency could be considered a non-invasive test to differentiate between patients at low and at high risk for tubal occlusion.	Conditional ⊕OOO

In patients at high-risk for tubal abnormality, visual demonstration of tubal patency is necessary.	GPP

Justification

The current evidence, consisting of a systematic review and meta-analysis including 23 cohort studies, and an additional 13 cohort studies that were published after the systematic review, showed CAT could be considered a non-invasive test to differentiate between patients at low and at high risk for tubal occlusion. However, although the techniques were not compared head-to-head, the sensitivity versus laparoscopy is lower compared to HSG and HyCoSy. The specificity seems to be good across different tests. It has to be noted that the validity of the test varies according to the assay used. This was investigated in a cohort study that reported a discrepancy in 21% of patients between MIF and ELISA assays for IgG (Gijsen et al., 2002). Moreover, CAT does not allow evaluation of the degree of occlusion or occlusions due to other infections than CT.

The reviewed data suggests a role (although limited) for CAT in clinical practice. Given the low false negative rate in testing, a negative result combined with low-risk medical history could be considered specific for tubal patency. Given the somewhat low PPV for the CAT, both a positive test as well as a negative test combined with a high-risk medical history should be confirmed with visual methods like HyCoSy, HSG or laparoscopy depending on the assessments needed. To highlight the role for medical history, Hubacher *et al.* reported tubal pathology (confirmed by laparoscopy) in 84.3% of patients with a high-risk medical history (based on a logistic regression model using past pelvic inflammatory disease symptoms, previous history of a lower genital tract infection, previous vaginal discharge, and antibodies to Chlamydia trachomatis) (Hubacher et al., 2004). Since the systematic review by Mol *et al.*, newer antibodies and more specific CAT have emerged with improved performance of these tests, however, limitations especially with sensitivity still remain (Horner et al., 2021).

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.4).

Table 3: Accuracy of Chlamydia antibody testing compared to gold standard laparoscopy and dye for tubal patency testing. IF: immunofluorescence, MIF: microimmunofluorescence. True and false positive and true and false negative data were extracted from the included publications followed by calculations on sensitivity, specificity, predictive values and likelihood ratios by the GDG. PPV: positive predictive value, NPV: negative predictive value, LR: likelihood ratio.

Reference	Method	No of patients	Clinical background	True positive	False positive	True negative	False negative	Sensitivity	Specificity	PPV	NPV	LR+	LR-
(Babay and Al-Meshari, 1993)	Iodine stain	75	History of urinary tract infection, history of PID, history of previous pelvic surgery was recorded	33	16	21	5	0.87	0.57	0.67	0.81	2.01	0.23
(Akande, et al., 2003)	IF	434	Not specified	358	192	380	76	0.82	0.66	0.65	0.83	2.46	0.26
(Sönmez et al., 2008)	IF	152	No patients with history of pelvic surgery, endometriosis, tuberculosis	18	18	62	27	0.40	0.78	0.50	0.70	1.78	0.77
(Veenemans and van der Linden, 2002)	IF	277	Women with only one tube or tuboperitoneal abnormality not caused by CT	28	50	60	7	0.80	0.55	0.36	0.90	1.76	0.37
(den Hartog et al., 2004)	MIF	313	No previous pelvic surgery	32	20	234	27	0.54	0.92	0.62	0.90	6.89	0.50
(den Hartog et al., 2005)	MIF IgG	313	No previous pelvic surgery	32	20	234	27	0.54	0.92	0.62	0.90	6.89	0.50
	EIA IgA			21	21	233	38	0.36	0.92	0.50	0.86	4.31	0.70
(Ng et al., 2001)	MIF	110	No history of any pelvic surgery	17	11	68	14	0.55	0.86	0.61	0.83	3.94	0.52
(Logan, et al., 2003)	EIA	207	No previous laparoscopy or tubal surgery	23	17	127	40	0.37	0.88	0.58	0.76	3.09	0.72
(Rantsi et al., 2019)	EIA TroA IgG	116	No prior pelvic surgery	17	11	40	11	0.61	0.78	0.61	0.78	2.81	0.50
	EIA HtrA IgG			16	11	40	12	0.57	0.78	0.59	0.77	2.65	0.55
	ELISA MOMP IgG			15	17	34	13	0.54	0.67	0.47	0.72	1.61	0.70
(Coppus, et al., 2007)	ELISA	207	No previous tubal testing or surgery	23	17	127	40	0.37	0.88	0.58	0.76	3.09	0.72
(Singh et al., 2016)	ELISA	200	Not specified further	10	0	150	40	0.20	1.00	1.00	0.79	N/A	0.80
(Tanikawa et al., 1996)	ELISA	131	No previous pelvic surgery	24	27	60	20	0.55	0.69	0.47	0.75	1.76	0.66
(van Dooremalen et al., 2020)	ELISA	890	Not specified further	44	75	710	61	0.42	0.90	0.37	0.92	4.39	0.64

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II.5 Uterine factor

PICO Question: Which diagnostic procedures should be performed to confirm a normal uterine structure/anatomy, uterine wall/myometrium?

3D Ultrasound vs. 2D ultrasound

Evidence

In a prospective cohort study, 117 women were examined with 2D and 3D ultrasound (US) to detect the most common congenital uterine anomalies. In the study, distinction was also made between an initial 2D-US and expert 2D-US. In the overall diagnosis of uterine anomalies, 3D-US was found to be a significantly better technique than both initial and expert 2D-US. Accuracy of 3D-US was 97.1% versus 51.4% for initial 2D-US and 82.9% for expert 2D-US, compared with combined hysteroscopy and laparoscopy (Ludwin et al., 2013).

In a prospective cohort study, 108 women with suspected congenital Mullerian abnormalities were evaluated with 2D- and 3D-US. Compared to 2D-US, the sensitivity and specificity of real-time 3D-US were significantly higher in both the follicular phase (3D-US sensitivity 94.7% and specificity 75% vs. 30.2% and 78.1%, respectively) and the luteal phase (3D-US sensitivity 100% and specificity 93.7% vs. 42.1% and 81.2%, respectively). In the follicular phase, PPV for 3D-US was 90% vs. 76.6% for 2D-US and NPV 85.7% vs. 32%. In the luteal phase, PPV for 3D-US was 97.4% vs. 84.2% for 2D-US and NPV 100% vs. 37.1% (Caliskan et al., 2010).

In a prospective cohort study, 2D- and 3D-US were compared for the assessment of uterine anatomy and detection of congenital anomalies in 61 women with a history of recurrent miscarriage or infertility and who had previously been investigated by HSG. In 95.1% good quality images were obtained by 3D-US. In comparison with 2D-US, 3D-US had 98% specificity (vs. 88% for 2D-US), 100% sensitivity (vs. 94%), 100% PPV (vs. 97%) and 94% NPV (vs. 75%) (Jurkovic et al., 1995).

Recommendation

Ultrasound, preferably 3D, is recommended to exclude uterine anomalies in women with unexplained infertility.	Strong	⊕OOO

Justification

Current evidence comparing 2D- to 3D-US is limited. Despite 2D-US is shown to be a valid diagnostic tool to exclude uterine anomalies, 3D-US showed superior results. Furthermore, comparing 2D- and 3D-US, cost per scan are the same, and the test is not more painful or more invasive for patients. The GDG acknowledges that 3D-US may not be available in every clinic.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.5.1).

MRI

Evidence

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

Recommendation

MRI is not recommended as a first-line test to confirm a normal uterine structure and anatomy in women with unexplained infertility.	Strong	⊕OOO

Justification

We have found no evidence on the usefulness of MRI as a first-line test in confirming a normal uterine structure. Furthermore, MRI is expensive and time-consuming and should therefore be considered as a second-line diagnostic tool for the diagnosis of specific conditions.

PICO Question: Which additional diagnostic procedures should be performed to confirm an anatomically normal uterine cavity?

Evidence

A RCT including 678 asymptomatic subfertile women with normal 2D-US and no previous hysteroscopy were assessed for uterine pathologies in an office hysteroscopy prior to their first IVF treatment. The study reported 11% of women with normal 2D-US having abnormal findings on hysteroscopy; 6% had polyps (only 1 case with polyp >1cm), 2% intrauterine adhesions, 2% septa, 1% myomas. The authors concluded that the second-line hysteroscopy findings were few. Moreover, given the cost for routine hysteroscopy versus for example HyCoSy that is also widely available, they did not recommend routine hysteroscopy as a second-line assessment (Fatemi et al., 2010).

A retrospective study investigated 1726 infertile women with normal uterine cavity on 2D-US with subsequent office hysteroscopy for uterine abnormalities. 15.1% of all women had intrauterine lesions; 6% polyps, 5.7% adhesions, 1.5% isthmocele, 0.5% unicornuate uteri, 0.5% endometritis, 0.2% myoma, and 0.1% septum. History of abnormal uterine bleeding or previous dilatation and curettage were indicative for uterine abnormality in hysteroscopy (Yang et al., 2019).

A prospective cohort study evaluated the usefulness of hysteroscopy in 2017 infertile women who had been previously investigated to have normal uterine cavity on US or HSG. 31.8% of women had intrauterine lesions on hysteroscopy: 12.9%, septum, 12%, polyps, 5.5% submucosal myoma, and 1.4% adhesions (Bakas et al., 2014).

A prospective cohort of 100 women with UI (confirmed ovulation, patent tubes, normal semen analysis) were assessed for uterine abnormalities. After confirming assessment of the uterine cavity with 2D transvaginal US, 93% of women went through hysteroscopy. 86% of the women had some abnormality detected in the cervix, endometrium, or uterine wall. The most common finding was an endometrial polyp (31%) or hyperplasia (15%). The NPV for 2D-US for endometrial polyp was 0.84, for submucosal myoma 0.97, whereas PPV for these were as good as 1.0. compared to hysteroscopy. 2D-US was able to detect the thick endometrial lining in cases with polyp or hyperplasia (Makled et al., 2014).

A retrospective cohort study investigated in 294 women the value of routine HyCoSy with saline as contrast agent after normal 2D-US finding compared to targeted HyCoSy with saline. The study group consisted of 124 women with normal US finding whereas the control group consisted of 170 women with reported uterine abnormality in 2D-US. 10.4% of women with normal US finding showed a uterine abnormality in HyCoSy. However, only 23% of these were confirmed in hysteroscopy and none of these were confirmed by pathology. As for targeted investigations, 67.7% of abnormal HyCoSy findings were confirmed in hysteroscopy and of these 83.3% were further confirmed by pathology (Almog et al., 2011).

Recommendation

If ultrasound assessment of the uterine cavity is normal, no further evaluation is needed.	Strong	⊕OOO

Justification

Even though there were some additional uterine findings in subsequent hysteroscopy or HyCoSy procedures in women with normal ultrasonography findings, most of the diagnosis were polyps or a septum that likely will not have major effect on pregnancy outcomes especially if they present without any symptoms. The reproductive outcomes were, indeed, described in the Cochrane review that was not able to show any benefit for routine hysteroscopy (Kamath et al., 2019). None of the studies introduced 3D as one option and given that some of the papers were already quite old, the ultrasonography technology may not represent the latest 2D/3D performance. 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.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.5.2)

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II.6 Laparoscopy

PICO QUESTION: SHOULD WOMEN UNDERGO A LAPAROSCOPY BEFORE BEING DIAGNOSED WITH UI?

Evidence

In a retrospective cohort study of patients with a normal HSG or suspected unilateral pathology on HSG and in whom both HSG and laparoscopy were performed, the diagnostic benefit of laparoscopy was assessed. Among 63 patients who were assigned to ovulation induction and IUI, 60 patients were found to have laparoscopic findings that did not necessitate any change in the original treatment plan. In three patients (4.8%), abnormalities discovered at laparoscopy were of such an extent that a change in the original treatment plan and referral to IVF was needed. The conclusion of the authors was that laparoscopy may be omitted in women with normal HSG or suspected unilateral tubal pathology on HSG, since it was not shown to change the original plan indicated by HSG in 95% of the patients (Lavy et al., 2004).

Another study evaluated the accuracy of diagnostic laparoscopy prior to IUI and included 495 women with a normal HSG. In 124 women (25%), the laparoscopy changed the initial treatment plan of IUI. Of these 21 (4%) had severe abnormalities that resulted in a change to in vitro fertilisation or open surgery. The remaining 103 (21%) patients had fertility enhancing surgical interventions. However, because it is unclear if treating the abnormalities such as minimal and mild endometriosis or milder adhesions increases success of subsequent IUI, the authors recommended a randomised trial (Tanahatoe et al., 2003).

The same group later published a randomized controlled trial including 154 women with UI. In this RCT, 77 women were assigned to have a diagnostic laparoscopy performed with ablation or resection of stage I/II endometriosis lesions or adhesiolysis if needed before IUI and 77 women were assigned to have six cycles of IUI followed by diagnostic laparoscopy in case of no pregnancy. The overall pregnancy rate was not significantly different between 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. There were no complications as a result of laparoscopy (Tanahatoe et al., 2005).

Recommendation

Routine diagnostic laparoscopy is not recommended for the diagnosis of unexplained infertility.	Strong	⊕OOO

Justification

Although different tests exist to reliably detect tubal patency, laparoscopy is the only method for directly visualising the pelvic anatomy and diagnosing peritoneal factors such as minimal or mild endometriosis or subtle tubal abnormalities. The presence of subtle tubal abnormalities was reported to be present in 103 of 208 patients (54.3%) with infertility and consisted of anatomical lesions such as tubal diverticula, Morgagni hydatids, accessory fallopian tubes, tubal phimosis, agglutination and sacculation (Guan and Watrelot, 2019). However, it is unknown to what extent these abnormalities contribute to infertility and there are no randomised trials that address the effectiveness of correction of these subtle lesions. The trial by Tanahatoe et al (2005) was small but showed no benefit of a diagnostic laparoscopy before treatment of UI in women with proven patent tubes on HSG (Tanahatoe et al., 2005).

One might argue that some patients would like to have a diagnostic laparoscopy to exclude all pelvic pathology, even though there is limited or no clinical benefit. Considering the fact that a diagnostic laparoscopy is not risk-free and requires a dedicated theatre team, general anaesthesia and operating time, time off work for the patient, costs are higher than the benefits. A formal cost-effectiveness analysis has not been performed.

Routine laparoscopy is not recommended in infertile women at low risk for tubal pathology but should be reserved for women with an abnormal HSG or those at risk for tubo-peritoneal disease due to a history of PID, previous ectopic pregnancy or clinically suspected or known endometriosis.

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II.7 Cervical/ vaginal factor

PICO QUESTION: WHAT IS THE NEED FOR FEMALE LOWER GENITAL TRACT INVESTIGATIONS?

POST-COITAL TEST (PCT)

Evidence

A systematic review and meta-analysis, including 4007 women from 11 studies, reported that the predictive values of normal and abnormal post-coital test (PCT) were 0.37-0.92 and 0.58-0.85 respectively. Sensitivity ranged between 0.10 and 0.90 and specificity ranged between 0.30 and 0.97. Likelihood ratios for normal and abnormal PCT were 0.77 and 1.85 respectively (Oei et al., 1995).

In a randomised controlled trial, 444 women were randomised to undergo a PCT or not. Fertility treatments were given more often in the intervention group compared to the control group (54% vs. 41%). However, cumulative pregnancy rates at 24 months were similar with and without PCT (49% (42-55%) vs. 48% (42-55%) (Oei et al., 1998).

In a retrospective cohort study, including 2476 patients with UI, the long-term overall pregnancy rates after a positive or a negative PCT were compared. The spontaneous and overall (OI, IUI, IVF) pregnancy rates were 37.7% and 77.5%, respectively, after a positive PCT which was significantly higher compared to 26.9% and 68.8% after a negative test (Hessel et al., 2014).

A retrospective study, including 200 couples who underwent a PCT as part of their routine fertility work-up, investigated the predictive value of normal and abnormal PCTs on pregnancy rates. The predictive values of normal and abnormal PCTs were 0.54 and 0.58 overall and 0.74 and 0.47 if only untreated women were considered. Sensitivity and specificity were, respectively, 0.47 and 0.65 for all women and 0.54 and 0.68 for untreated women only. Likelihood ratios for normal and abnormal PCTs were 0.83 and 1.32 overall and 0.67 and 1.72 in untreated women (Oei et al., 1996).

In a retrospective re-analysis of 207 couples originally studied between 1982 and 1983, it was found that in couples with less than 3 years of infertility and positive PCT, 68% conceived within 2 years compared with 17% of those with negative result. After 3 years of infertility, corresponding rates were 14% and 11% (Glazener et al., 2000).

Recommendation

The post-coital test is not recommended in couples with unexplained infertility.	Strong	⊕⊕○○

Justification

The meta-analysis showed that PCT has poor discriminating capacity. Cumulative pregnancy rates seem to be similar after a positive or a negative test. Importantly, it is an invasive test for the patient and does not change further management. Therefore, PCT is not recommended in infertility investigations.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.7).

Vaginal microbiota testing

Evidence

In a case-control study, gut and vaginal microbiota were compared between women with UI (n=10) and fertile controls (n=11). Firmicutes accounted for the vast majority of the vaginal bacteria, with higher relative abundance in UI than controls (69.7 vs. 53). *Fusobacteria* (18% vs.0.14) and *Bacteriodetes* (4.1% vs. 0.92) were relatively more abundant in the controls than in the UI group. Within the genus of *Lactobacillus, L. jensenii* and *L. vaginalis* were only detected in the UI group (Patel et al., 2022).

Another case-control study compared vaginal and endometrial microbiota between women with UI (n=26) and fertile controls (n=26). In the vaginal samples, the lactobacilli-impaired microbiota proportion was significantly higher in women with UI compared to fertile women (76.9% vs. 26.9%). Similarly, the *Mycoplasma hominis* flora increment or pathogenic microorganism growth rate was significantly higher in women with UI compared to fertile women (34.6% vs. 7.7%). The amount of lactobacilli per total bacterial mass mean proportion in the vaginal samples was significantly lower in women with UI compared to fertile women (38.2% vs. 76.3%) (Sezer et al., 2022).

In a prospective cohort study, including 25 couples with UI, the association between vaginal microbiota and pregnancy outcome after IUI was investigated. Five out of 23 women achieved a clinical pregnancy, and this was associated with a more evident *Lactobacillus spp* domination, comparable to that observed in controls. Furthermore, a significantly lower Shannon index was found in pregnant women compared to non-pregnant women (0.8 ± 0.9 vs. 1.5 ± 1.1) (Amato et al., 2020).

In a prospective cohort study, including 47 (25 unexplained and 22 explained infertility) couples undergoing ART, the difference in vaginal microbiota between unexplained and explained infertility was investigated. There were no significant differences in alpha or beta diversity metrics between explained and unexplained infertility couples. In comparison with the unexplained group, there was a decrease in lactobacilli in the vaginal lavage of women with explained infertility (Campisciano et al., 2020).

In a case-control study, the microbiome of 96 cervical-vaginal samples (27 infertile women and 69 fertile controls) was compared. Compared to controls, the idiopathic infertility group showed the highest biodiversity of species (Simpson's reciprocal indexes, 1.5±0.5 vs. 2.43±1.19) (Campisciano et al., 2017).

In a case-control study, the incidence of bacterial etiological factors causing inflammation of the upper and lower reproductive tract in women treated for infertility with no clinical parameters of acute inflammation of the vagina and/or the cervix was assessed. Normal bacterial vaginal flora was confirmed in 80 women (79%) treated for infertility and 51 women (85%) from the control group. Bacterial vaginosis was confirmed (based on pH, Nugent score and quantitative culture results) in 7 women (7%) treated for infertility, and none from the control group (Tomusiak et al., 2013).

Recommendation

Vaginal microbiota testing could be considered in couples with unexplained infertility only in a research setting.	Research only

Justification

It is very difficult to compare studies investigating the role of vaginal microbiota, due to the different detection methods used (wet mount microscopy, modified Spiegel criteria, Nugent scores, qPCR assays for common bacteria in bacterial vaginosis). Additionally, the vaginal swabs were taken at different time points of the menstrual cycle in different studies (before and during ovarian stimulation, at oocyte retrieval or at embryo transfer). The lactobacillus dominance is also defined differently between papers. There is currently insufficient evidence of a role of abnormal vaginal microbiota in UI. Furthermore, there is currently no evidence suggesting that correcting abnormal vaginal microbiota improves fertility outcomes.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.7).

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II.8 Male genito-urinary anatomy

PICO QUESTION: SHOULD MEN UNDERGO ADDITIONAL DIAGNOSTIC PROCEDURES TO CONFIRM NORMAL GENITO-URINARY ANATOMY BEFORE BEING DIAGNOSED WITH UI?

Evidence

Mean testicular volume (TV) was positively associated with sperm concentration (r=0.315, p<0.0001 unadjusted, r=0.274 p<0.0001 after adjustment for confounding factors) and total sperm count (r=0.219, p=0.001 unadjusted, r=0.278 p<0.0001 after adjustment for confounding factors). Subjects with testicular inhomogeneity (defined as an echotexture score, ranging from 0 (regular pattern) to 5 (tumour suspected)) showed a lower sperm vitality compared with the rest of the sample, while those with any parenchymal calcification had lower sperm concentration and total count. Intratesticular artery peak systolic velocity was positively associated with sperm normal morphology (r=0.226, p=0.017 unadjusted, adjusted r=0.240 p<0.008). Epididymal mean head size was positively associated with normal sperm morphology (r=0.385, p<0.0001, adjusted r=0.233, p=0.002) and vas deferens mean size was positively associated with progressive motility (r=0.214, p=0.004, adjusted r=0.235, p=0.001). Subjects with a Mixed antiglobulin reaction (MAR) test ≥ 1% showed a higher prevalence of epididymal tail echotexture inhomogeneity (OR 5.75, 95% CI 1.35-24.1), and a higher mean size of vas deferens and of epididymal body and tail, as compared with the rest of the sample (Lotti et al., 2021).

Recommendation

Testicular imaging is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○

Justification

There is no additional benefit on performing scrotal colour Doppler ultrasound (CDUS) on male partners with normal semen parameters and having undergone physical examination. CDUS may aid physical examination in assessment of ultrasound patterns of testicular anatomy and structure. It can thus be helpful to identify scrotal abnormalities and to assist in better understanding the pathophysiology of sperm abnormalities and male infertility. Though CDUS shows some association with semen parameters, it would not be beneficial to replace gold standard WHO semen analysis for CDUS.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.8).

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II.9 Male additional tests

PICO QUESTION: IS THERE ADDED VALUE OF ADDITIONAL TESTS IN THE MALE WITH NORMAL WHO SEMEN ANALYSIS?

The GDG acknowledges that presently unexplained infertility (UI) is a diagnosis by exclusion. The GDG supports the urgent need to develop robust standardised methods for establishing etiological diagnosis in the male partner (Barratt et al., 2017, Björndahl, 2022). In some patients underlying sperm dysfunction may not be detected during a routine semen assessment of the ejaculate, which may appear to be normal regarding basic semen parameters (WHO, 2021). GDG considers that some adverse reproductive outcomes that have been identified during medical and reproductive history examination may indicate the use of further tests procedures specified in the WHO 6th ed chapters 3 and 4 to assess sperm function. However, these tests have a research status only or are not regarded for routine use in clinical practice until sound evidence is developed (WHO, 2021).

ANTI-SPERM ANTIBODIES (ASA)

Evidence

A case-control study investigated the effect of anti-sperm antibodies (ASA) in 1060 normozoospermic infertile men with female partners with no abnormalities found after full investigation and 107 normozoospermic fertile men (control group). Significantly more ASA was found in the infertile group compared to the controls (Mixed antiglobulin reaction (MAR) ≥50% in 15.6% (166/1060)) vs. 1.9% (2/107) and the mean ASA titre was higher. The relative infertility risk for MAR ≥50% was 8.38, which increases starting from MAR-IgG >25%. Also, in ASA-positive men, acrosome reaction was decreased, DNA fragmentation increased and higher reactive oxygen species (ROS) were found (Bozhedomov et al., 2015).

In a cohort study, including 84 men with positive MAR test, the occurrence of natural pregnancies and the effectiveness of IUI were analysed in connection with the degree of sperm autoimmunisation. 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)) (Barbonetti et al., 2020).

In a small case-control study pregnancy rates were compared in IVF couples with ASA positive males and couples without ASA. Pregnancy rates were not significantly different between couples with ASA positive males (11% (1/9)) and couples without ASA (44% (4/9)) (Vazquez-Levin et al., 1997).

In a cohort study, men with anti-sperm antibodies (MAR assay) undergoing ICSI or conventional IVF were compared to an ICSI control group with male infertility without anti-sperm antibodies. Clinical pregnancy rates were 46% with ICSI (13/28), 30% (11/37) with conventional IVF and 30% (6/20) in the control group. Five miscarriages occurred in the ICSI group, compared to three in the IVF and none in the control group (Lähteenmäki et al., 1995).

In a cohort study, pregnancy rates were compared between females with ASA and males with ASA on their sperm. Overall pregnancy rate in females with ASA was 9/15 and 7/16 in males with ASA.

Pregnancy rate in males with high % ASA (≥50%) was 38% and in low % ASA (<50%) was 50% (Pagidas et al., 1994).

In a cohort study, IVF pregnancy rates were compared between couples with ASA positive males and couples without ASA. Pregnancy rate/embryo transfer was not significantly different between ASA positive (46.1%) and ASA negative couples (33.3%) (Rajah et al., 1993).

In a cohort study couples were divided into three categories according to their sperm MAR test results, and fertilisation and pregnancy rate (per embryo transfer) were compared in those groups (weakly positive, >0 and <40%; positive, >40 and <90% ; strongly positive, >90%). Pregnancy rate as per MAR category were not significantly different: 43% versus 45% versus 33% (Lähteenmäki, 1993).

In a cohort study, couples with UI were divided into couples with >50% sperm antibody bound and couples with <50% sperm antibody bound. There was a significant higher pregnancy rate found in couples with <50% sperm antibody bound, 66.7% (6/9) compared to 15.3% (4/26) in couples with >50% sperm antibody bound (Ayvaliotis et al., 1985).

Recommendation

Testing for anti-sperm antibodies in the semen is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕OOO

Justification

There is insufficient evidence to suggest the benefit of anti-sperm antibodies tests in couples with UI. The quality of data is very low (old and underpowered studies, some with old methodologies, methods not following standard procedures, not sufficient or lacking inclusion criteria, most of them analysing couples undergoing MAR). Cut-off values are inconsistent and differ between studies, thresholds are not validated. Furthermore, currently there are no evidence-based reference values for antibody-bound spermatozoa in the MAR test of semen from fertile men (WHO, 2021). In some of the available studies male partners had different degrees of abnormal semen parameters, including impaired motility which can influence the validity of ASA tests (tests should be performed on motile spermatozoa).

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.9).

DNA FRAGMENTATION TEST

It is important to emphasize that sperm DNA fragmentation (SDF) assays cannot be used interchangeably as they have often been in literature. Each SDF assay currently used in clinical practice is based on specific technical methodology which defines its capacity to measure different structural aspects of sperm DNA damage (WHO, 2021).

Evidence

In a prospective cohort study including couples undergoing their first ICSI cycles for UI, the effect of sperm DNA fragmentation (SDF; by acridine orange test) on reproductive outcomes was investigated. Cumulative live birth rate was significantly higher in the low versus high SDF group (60.8% (59/97) vs. 41.7% (20/48)). Subgroup analysis by fresh or frozen embryo transfer showed that live birth was significantly different between groups with fresh embryo transfer (ET), but no difference was found with frozen ET (Repalle et al., 2022).

In a prospective cohort study, including couples undergoing their first IVF with ICSI cycle, the influence of SDF (by sperm chromatin dispersion test) on reproductive outcomes was investigated. A significantly higher miscarriage rate (17.8% vs. 39.9%) was observed in cycles with SDF above the cut-off (30%), however, there was no difference in clinical pregnancy rate (32.4% vs. 30.3%) (Borges et al., 2019).

In a retrospective cohort study, including couples with UI and poor IUI outcome, couples were assigned to either IVF or ICSI, based on the results of their SDF testing (sperm chromatin structure assay (SCSA) or TUNEL). Thirty-one couples with normal sperm DNA fragmentation underwent IVF, resulting in a clinical pregnancy rate of 12.7%. The remaining 343 couples underwent ICSI, resulting in a cumulative pregnancy rate of 18.7% (O'Neill et al., 2018).

Recommendation

Testing for sperm DNA fragmentation is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○

Justification

Data from meta-analyses in recent years indicate that SDF may adversely affect reproductive success in natural and assisted conception (Osman et al., 2015, Simon et al., 2017, Sugihara et al., 2020, Tan et al., 2019). Some evidence suggests increased sperm DNA damage in infertile men compared to fertile men (Evenson et al., 1999, Spanò et al., 2000), and similar degree of SDF in ejaculates from infertile men with abnormal semen parameters and infertile men with semen parameters in the reference range (Saleh et al., 2002).

Several meta-analyses have shown that different SDF assays have different predictive accuracy for pregnancy and each assay had a different predictive value for IVF and ICSI (Cissen et al., 2016, Zhao et al., 2014). Furthermore, each test has different clinical thresholds validated by the laboratories performing the test. The methodology and the cut-offs are not standardized worldwide, nor is there a consensus on which test is preferred. Differences in cut-offs between available studies might falsely re-categorize patients into those at risk of adverse reproductive outcome. Clinical relevance for performing SDF test in couples with UI is questionable since available data on reproductive outcomes (pregnancy rate, live birth rate, miscarriage) is predominantly based on couples undergoing IVF/ICSI. The quality and heterogeneity (including parental age and previous MAR treatments) of the available data from three cohort studies does not allow conclusive recommendation on the benefit of performing SDF testing in males from couples with UI who have normal semen parameters. Published studies so far have not directly tested the effect of SDF testing on the clinical management of infertile couples (i.e., that the fertility outcomes of those who had testing are different from those who did not), which does not support a recommendation for its routine use in the initial evaluation of the male. Upcoming evidence might show benefit of specific tests, when validated.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.9).

SPERM CHROMATIN CONDENSATION TEST

Evidence

No studies were identified to answer this PICO question.

Recommendation

Sperm chromatin condensation test is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕OOO

Justification

Abnormal sperm chromatin structure may cause sperm DNA damage, such as double or single DNA strand breaks, because of poor chromatin condensation (i.e., defects in histone replacement by protamines) (WHO, 2021). Abnormal sperm chromatin remodelling has been detected in infertile men (Zhang et al., 2006). Depending on the assay used for DNA fragmentation, some studies have shown an association between abnormal sperm histone retention and/or protamination anomalies and sperm DNA fragmentation in infertile men with abnormal semen analysis (Simon et al., 2014, Tavalaee et al., 2009, Torregrosa et al., 2006).

Currently, we found no published evidence to suggest the clinical significance of routine sperm chromatin condensation testing in men with UI. Published studies either did not assess men with UI, assessed the test inappropriately in relation to clinical outcomes, or the sperm chromatin condensation test was evaluated together or in association with other methods for sperm quality. Furthermore, published studies so far have not directly tested the effect of sperm chromatin condensation testing on the clinical management of infertile couples (i.e., that the fertility outcomes of those who had testing are different from those who did not) which does not support a recommendation for its routine use in the initial evaluation of the male. Instead, sperm chromatin condensation is usually assessed in studies that investigate its associations with aneuploidy and other methods to evaluate sperm chromatin integrity (i.e., SDF). Thus, there is not enough strong evidence to suggest that assessing sperm chromatin structure integrity solely by sperm chromatin condensation test can be reliably predictive of reproductive outcomes or how it can be used to guide clinical decision making (Barratt et al., 2010).

SPERM ANEUPLOIDY SCREENING

Evidence

No studies were identified to answer this PICO question.

Recommendation

Sperm aneuploidy screening is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕OOO

Justification

At least 15% of human male factor infertility can be attributed to genetic factors that underlie the major categories of male infertility – spermatogenic quantitative defects, ductal obstruction or dysfunction, hypothalamic–pituitary axis disturbances, and spermatogenic qualitative defects (reviewed in (Krausz and Riera-Escamilla, 2018)). Azoospermia is the aetiological category with the highest frequency of known genetic factors (25%) contributing to male infertility (Krausz and Riera-Escamilla, 2018), but the number of identified genes linked with other seminal phenotypes and male infertility aetiological categories is constantly expanding (Houston et al., 2021, Riera-Escamilla et al., 2022). The risk of men being carriers of genetic anomalies progressively decreases with increasing sperm output (Krausz, 2011).

The incidence of sperm aneuploidy is rare in fertile men (WHO, 2021). Abnormal levels of aneuploid sperm are most commonly observed in men with spermatogenic failure, oligozoospermia or oligoasthenozoospermia, and among normozoospermic men who are partners in couples with recurrent pregnancy loss (Ramasamy et al., 2015, WHO, 2021). Thus, based on current state-of-the-art knowledge about prevalence and male infertility aetiologies underlined by chromosomal abnormalities, aneuploidy is not indicated for routine testing in men with normal semen parameters.

HORMONAL TESTING

Evidence

No studies were identified to answer this PICO question.

Recommendation

Serum hormonal testing is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕OOO

Justification

Semen analysis as performed according to WHO Laboratory Manual for the Examination and Processing of Human Semen standards (WHO, 2021) is used to assess male reproductive function and genital tract patency. In this context, semen analysis is the cornerstone in the evaluation of the reproductive hormonal status for men. In cases of abnormal sperm parameters (oligozoospermia and azoospermia), potential hypogonadism is ruled out by reproductive hormone (testosterone and gonadotropins) testing which provide a functional readout of the hypothalamic-pituitary-testicular axis. Thus, the hormonal profile will be helpful in following extended examination to accurately diagnose underlying pathological conditions associated with abnormal semen parameters. However, no evidence was found supporting endocrine testing as a first line of investigation for males with UI and results from a basic semen examination in the reference range according to the WHO criteria.

Currently, endocrine testing is not recommended as a primary first line of investigation for males with UI and results from a basic semen examination in the reference range according to the WHO criteria (Minhas et al., 2021, Schlegel et al., 2021, WHO, 2000). However, given the essential role of FSH for the initiation and maintenance of full spermatogenesis, increasing research emphasis has been given to single nucleotide polymorphisms in FSH beta (FSHB) and FSH receptor (FSHR) genes and their effects on male infertility (Ferlin et al., 2011, Grigorova et al., 2011, Schubert et al., 2019, Schubert et al., 2022, Tamburino et al., 2017a, Tamburino et al., 2017b, Tüttelmann et al., 2012).

Human Papilloma virus (HPV)

Evidence

No studies were identified to answer this PICO question.

Recommendation

HPV testing of semen is not recommended when conventional semen analysis according to WHO criteria is normal.	Strong	⊕OOO

Justification

There are over 200 HPV subtypes and most people will test positive for a HPV type at some point during life. Furthermore, HPV is a transient infection which most often clears spontaneously, but it is unknown how fast infectious HPV is cleared in males and females (Depuydt et al., 2019, Giuliano et al., 2011). There is some evidence showing reduced pregnancy rates in donor IUI cycles and autologous IUI cycles with moderate male factor infertility in HPV positive semen versus HPV negative (Depuydt et al., 2018, Depuydt, et al., 2019). Conflicting data on association between semen HPV presence and alteration of sperm parameters (Luttmer et al., 2016), as well as the effects of semen HPV infection on reproductive outcomes makes it impossible to recommend routine screening of HPV in a diagnostic setting in assisted reproduction. Therefore, HPV testing could be discussed with couples scheduled for an IUI cycle only in research settings. Further information on the management of HPV in couples undergoing MAR can be found in the ESHRE guideline “Medically assisted reproduction in patients with a viral infection or disease” (Eshre Guideline Group on Viral infection/disease, 2021).

Microbiology test

Evidence

No studies were identified to answer this PICO question.

Recommendation

Microbiology testing of semen is not recommended when semen analysis according to WHO criteria is normal.	Strong	⊕○○○

Justification

Excessive numbers of leukocytes in the ejaculate (leukocytospermia, pyospermia) may be associated with infection and poor sperm quality (WHO, 2021). However, in the case of a normal physical examination and in the absence of symptoms associated with genitourinary tract infection, medical and reproductive history do not give indications for signs of infection, further microbiological culture of the semen is not usually warranted.

Tests for discriminating specific leukocyte types from round immature germ cells are not part of the routine semen analysis according to the latest, sixth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. These techniques are included in the 'extended examination' section of the manual. However, the clinical value of these specific tests is not clear and there are currently no evidence-based reference values for these tests in semen of fertile men (WHO, 2021).

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II.10 Additional tests for systemic conditions

PICO Question: Should there be additional evaluations of possible systemic cause of UI in the couple?

Auto-immunity

Evidence

Anti-sperm antibodies (ASA) in serum

A study, including 42 couples with unexplained infertility (UI), investigated the presence of serum anti-sperm antibodies (ASA) and their association with UI. The prevalence of ASA was 14.3% (6/42) among all couples, 9.5% (4/42) among males and 4.8% (2/42) among females. Of the 42 couples, 22 couples were managed with IVF-ICSI, and it was found that no relation between ASA status and the successfulness of IVF-ICSI exists (Yasin et al., 2016).

In a study, including 30 men and 24 women with UI, presence of ASA was compared to fertile controls (45 men and women) and their association with UI was investigated. With the indirect immunofluorescence technique 22/54 patients tested positive for ASA and 3/45 controls. With ELISA, 20/54 patients tested positive and 4/45 controls. This suggested a strong correlation between UI and ASA (Monem and Moalla, 2003).

A study including 44 couples where the only detectable cause of infertility was the presence of ASA in the female, looked at pregnancy rates after IVF in these couples. After IVF, fertilisation rates were slightly lower in couples where the only detectable cause of infertility was the presence of ASA in the female than in patients with other infertility aetiologies but was successful in 45% without the need for ICSI (Mardesic et al., 2000).

In a study, including 698 couples with UI, prevalence of ASA was investigated, and their impact on pregnancy rates. In the study 16.5% of the men and 21.6% of the women had serum ASA. The overall incidence of immobilising antibodies was 5.6% for men and 6.4% for women. In men, the pregnancy rate dropped significantly from 42.7% to 7.1% at high agglutinin titers >1:16. In women at high titers ≥1:16 the incidence of pregnancy was only 4.0%, compared with 46.2% in the negative group (Menge et al., 1982).

Female coeliac disease

In a systematic review and meta-analysis, the risk of coeliac disease in UI was investigated in 586 patients and 5088 controls without risk factors, from 7 case-control studies. The OR for coeliac disease was 5.06 (95% CI 2.13–11.35) in patients with UI (Tersigni et al., 2014).

A small cohort study in 65 couples with UI also investigated the role of coeliac disease in UI. Overall, 7.9% of patients tested positive for antigliadin, anti-endomysial or tissue transaminase antibodies. In these cases, an intestinal biopsy was performed, however, only one male and one female tested positive for coeliac disease (Karaca et al., 2015).

Thyroid antibodies

In a prospective cohort study, 69 patients with UI were screened for anti-thyroid antibodies and their effect on IVF outcome. Patients were divided in 3 groups, group 1 consisted of infertile patients without thyroid pathology (n=31), group 2 consisted of infertile patients with normal thyroid function and anti-thyroid antibodies (n=23), and group 3 consisted of infertile patients euthyroid by medical therapy and anti-thyroid antibodies (n=15). Clinical pregnancy rate was significantly lower in thyroid antibody positive groups (groups 2 and 3) compared to controls (30.4% vs. 13.3% vs. 41.9% respectively) (Kilic et al., 2008).

A small cross-sectional study, including 14 women with UI, reported no cases of subclinical hypothyroidism and 3/14 (21.4%) women with thyroid antibodies, which was not significantly different from findings in the control group (Abalovich et al., 2007).

In another cross-sectional study including 73 patients with UI and 100 controls (randomly selected, parous women) thyroid dysfunction and auto-immunity in infertility was investigated. The percentage of patients with positive thyroid peroxidase-antibodies (>100 kU/L) was 7% in UI patients, which was not significantly different from controls (8%) (Poppe et al., 2002).

<u>Other auto-immune tests</u>
A number of publications were identified examining other auto-immune tests, such as anti-endometrial antibodies (Palacio et al., 1997), anti-ovarian antibodies (Luborsky et al., 2000), zona pellucida antibodies (Hovav et al., 1994) or combinations of several auto-immune tests (Aoki et al., 1995, Bellver et al., 2008, Kovács et al., 2012, Luborsky et al., 1999, Radojcić et al., 2004, Witkin et al., 1984). However, data was too sparse to draw conclusions.

Recommendation

Testing for anti-sperm antibodies in serum of either males or females with unexplained infertility is not recommended.	Strong	⊕OOO

Testing for coeliac disease in women with unexplained infertility can be considered.	Conditional	⊕⊕OO

Testing for thyroid antibody and other autoimmune conditions (apart from coeliac disease) in women with unexplained infertility is not recommended.	Strong	⊕OOO

Justification

Current evidence indicates that the benefit of testing for ASA is low. However, the studies are relatively small and, apart from one, somewhat dated. Patients value explanations for UI but uncertainty about the evidence, no clear treatment shown to be effective and potential risks of treatment limit value of treatment. There is no clear benefit of ASA testing for health equity and the current evidence shows no feasibility of treatment in case of a positive test.

The benefits of testing for coeliac disease may be considerable depending on the test used. If blood testing for antibodies, the patients would value investigation at small cost with an easy intervention, i.e., a dietary change.

Testing for thyroid antibodies and other autoimmune diseases appears to have little benefit, but may reassure the patient that a full investigation has been implemented. Costs would be relatively small but in the absence of treatment, efficacy would be of little value.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.10).

THYROID HORMONES

Evidence

In a case-control study, the role of altered thyroid hormones in UI was investigated in 44 women with UI and 44 fertile controls. Both thyroid stimulating hormone (TSH) and T4 were found to be slightly higher in women with UI than controls (1.49±0.76 vs. 1.12±0.54 mIU/L and 10.48±1.89 vs. 9.18±1.53 (µg/dl) (Rehman et al., 2020).

In a cross-sectional study, the association between thyroid hormones and UI was investigated in 187 women with UI and compared with 52 women with male infertility. Median TSH levels were significantly higher in the UI group compared to the male factor infertility group (1.95 (IQR 1.54-2.61 vs. 1.66 (IQR 1.25-2.17) mIU/L. Also, more women with UI had levels >2.5mIU/L (26.9 vs. 13.5%). Thyroid peroxidase antibodies (TPO) were significantly lower in UI women compared to male factor infertility (13.3 (IQR 10.2-18) vs. 90.4 (IQR 18.4-2994.3) IU/mL) (Orouji Jokar et al., 2018).

In a case-control study, the prevalence of thyroid hormone and thyroid antibody abnormalities were investigated in 25 women with UI and 45 normal controls. The fT4 levels were significantly higher in the UI group compared to controls (1.14±0.13 vs. 0.88±0.11 pmol/L), and the fT3 was significantly lower (3.48±0.46 vs. 4.7±2.52 pmol/L). No difference in thyroid autoimmune antibodies (TAI) was found between groups (Duran et al., 2013).

In a cross-sectional analysis of a prospective cohort study, the role of thyroid hormone and thyroid antibody abnormalities in UI were investigated in 95 women with UI and compared to women with male factor infertility. There were no differences in the thyroid hormones but in the UI group, 86% were TAI negative and 14% positive, which was not significantly different from controls (Unuane et al., 2013).

Recommendation

TSH measurement is considered good practice in pre-conception care.	GPP

No additional thyroid evaluation in women is recommended if TSH is within the normal range.	Strong	⊕○○○

Justification

According to Endocrine Society guidelines (De Groot et al., 2012), TSH should be measured in pre-conception care.

The results of the studies were heterogenous but there is little evidence that additional thyroid testing is required despite widely available testing and cheap intervention with thyroid preparations. Additional measurement of thyroid antibodies does not appear to help further diagnosis of UI despite patient acceptance of simple thyroid replacement.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.10).

THROMBOPHILIA

Evidence

A small cross-sectional study, including 31 patients with UI and 32 controls, investigated the role of thrombophilia (anthrombin III deficiency, protein C and/or S deficiency, FVL, FII mutation, MTHFR C677T mutation, hyperhomocystinemia, Lupus anticoagulant) in UI. No significant differences were detected between UI and controls for any of the isolated or combined thrombophilia markers (Bellver et al., 2008).

In a large case-control study, 594 women with UI were screened for common prothrombotic polymorphisms (Factor V Leiden (FVL), prothrombin G20210A and activated protein C resistance (APCR)), and/or antiphospholipid antibodies (anticardiolipin IgG, beta 2 glycoprotein I antibodies, Lupus anticoagulant). APCR and/or FVL were significantly more prevalent in UI women vs. fertile women with previous spontaneous pregnancy (7.9% vs. 3.8%, OR 2.18, 95% CI 1.28-3.72). The prevalence of prothrombin G20210A or antiphospholipid antibodies was not different between the study group and fertile women (prothrombin 3.1% vs. 4.2%, OR 0.73, 95% CI 0.39-1.37; lupus/anticardiolipin 3.3% vs. 4.7%, OR 0.70, 95% CI 0.38-1.28). The presence of thrombophilia was not significantly associated with lower fertility success rate. Rather, women who had APCR and /or factor V Leiden or had antiphospholipid antibodies had significantly higher live birth rates in comparison to women who were tested negative (Steinvil et al., 2012).

In a case-control study, the frequency and SNP-SNP interactions between FVL G1691A, prothrombin G20210A mutation, and C677T MTHFR and PAI-1 4G/5G gene polymorphisms was evaluated in 105 women with UI and 120 fertile controls. Significant differences were found between UI women and controls only in the frequency of the MTHFR C677T CC genotype (19.1% vs. 40.8%), CT genotype (60% vs. 45.8%), and TT genotype (20.9% vs. 13.3%). Interaction of MTHFR plus FVL was associated with UI (Milenkovic et al., 2020).

In a case-control study, 230 women with UI and 240 fertile women were screened for the presence of congenital inherited thrombophilia (FVL mutation, prothrombin gene G20210A polymorphism, and deficiencies in protein S and C and AT). A significant higher prevalence of thrombophilia was found in women with UI compared to controls (13% vs. 7.1%). A significantly higher prevalence of prothrombin gene mutation was found in the UI group compared to controls (5.7% vs. 2.1%, OR 2.82, 95% CI 1.02-

8.03). The presence of FVL and anticoagulant protein deficiencies was not significantly higher (4.8% vs. 3.8% and 2.6% vs. 1.2%) (Fatini et al., 2012).

In a case-control study including 100 women with UI and 200 apparently healthy women without infertility and with previous term pregnancies, screening was performed for mutations of the FVL, G20210A in prothrombin, and of MTHFR C677 T. There was no significant difference between UI and control groups for any of the thrombophilia: MTHFR OR 1.28 (95% CI 0.68-2.4), Factor V Leiden OR 1.0 (95% CI 0.36-2.75), prothrombin OR 0.85 (95% CI 0.22-3.37) (Casadei et al., 2010).

In a case-control study, the frequency of inherited thrombophilia's (FVL G1691A, FVL H1299R (R2), factor II prothrombin G20210A, factor XIII V34L, b-fibrinogen -455G>A, PAI-1 4G/5G, HPA1 a/b (L33P), MTHFR C677T, and MTHFR A1298C) was investigated among 92 women with UI and 60 fertile control women. MTHFR C677T was the only gene to show a significant difference between women with UI (22%) and controls (0%) (Coulam and Jeyendran, 2009).

In a case-control study, 36 women with UI and 62 healthy, fertile controls were screened for the presence of thrombophilic mutations (FVL G1691A, MTHFR C677T, and FII G20210A). Significantly more mutations in FVL were found in the UI group compared to controls (30.6% vs. 0%), however, no significant differences were found in MTHFR or FII mutations (50% vs. 38.7% and 2.8% vs. 3.2%) (Behjati et al., 2006).

In a case-control study, 115 women with UI and 107 fertile women were screened for the PAI-1 4G -675 allele. A significant difference in allele frequency was found between UI and fertile controls. Of the UI group 22.6 % (26/115) was 5G/5G compared to 39.3% (42/107) of controls, and 77.4% (89/115) was either 4G/5G or 4G/4G compared to 60.7% (64/107) of controls (Kydonopoulou et al., 2017).

Recommendation

Testing for thrombophilia in women with unexplained infertililty is not recommended.	Strong	⊕○○○

Justification

All included studies are small and the results are heterogenous, the evidence is therefore considered of very low quality. Furthermore, comprehensive testing for thrombophilia can be expensive, given the multiple alleged effects. While patients may value thorough exclusion of thrombophilia, the cost is large compared to the potential benefit. Comprehensive testing may not be available globally, is expensive and affects accessibility. In the absence of any proof of effective treatment, investigation is of low value to a desired outcome.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.10).

OXIDATIVE STRESS

Evidence

Male

In a prospective cohort study the role of oxidative stress (DNA fragmentation, reactive oxygen species (ROS), malondialdehyde (MDA), protein carbonyl group (PC), nitrotyrosine (NT), total thiol group (SH) levels and ferric reducing antioxidant power (FRAP)) in 28 males with UI was investigated and compared to 14 fertile sperm donors. A significantly higher percentage of DNA fragmentation and ROS formation was found in the UI group compared to controls (72% vs. 4.2%; and 56% vs. 4.7% respectively). Furthermore, seminal plasma MDA, PC, and NT levels were significantly elevated in UI versus control group (8.6 vs. 5.2 nmol/ml, 0.78 vs. 0.46 nmol/mg protein and 234 vs. 148 nmol/L respectively) (Aktan et al., 2013).

In a prospective cohort study, the effects of DNA damage and oxidative stress (ROS, total antioxidant capacity (TAC), SCSA DNA damage) in semen were investigated in 23 males with UI and 16 fertile controls. No significant differences were found in isolated ROS or TAC between the UI group and controls. However, the ROS-TAC score was significantly lower in UI vs. control groups (47 (IQR 25th and 75th percentile 45-51) vs. 53 (IQR 50-58)). Also, the DNA fragmentation index (DFI) was significantly higher in the UI group compared to controls (23% (IQR 15-32) vs. 15% (IQR 11-21)). ROS negatively correlated with fertilisation (r=-0.59) and embryo quality (r=-0.89); DFI negatively correlated with fertilisation (r=-0.70) and embryo quality (r=-0.70) (Saleh et al., 2003).

In a case-control study, oxidative stress markers (ROS and lipid peroxidation, mitochondrial membrane potential, DFI, antioxidant capacity) were investigated in 23 men with UI and 34 fertile controls. Only ROS was significantly increased in UI males compared to fertile controls (121.2±29.9 vs. 71.7±8.7) (Mayorga-Torres et al., 2017).

In a case-control study, oxidative DNA damage to sperm cells was investigated in 30 males with UI and 22 fertile males. When comparing UI males with controls, seminal MDA (9.68±2.87 µM vs. 6.63±2.99 µM); serum MDA (12.55±3.17 µM vs. 7.7±2.37 µM), serum nitric oxide (NO; 19.26±7.81 µM vs. 11.18±5.61 µM), serum 8-OhdG/106dG (1.55±0.61 vs. 1.03±1.03) and leukocyte 8-OhdG/106dG (1.25±0.37 vs. 0.77±0.27) were significantly higher in UI males (Taken et al., 2016).

In a case-control study, ROS in semen were compared between 43 men with UI and 17 fertile controls. ROS was significantly higher in UI males compared to controls, both in neat and washed semen (0.79 (IQR 0.41-2.01) vs. 0.03 (IQR 0.014-0.11) 10^4 RLU/min/20 million sperms and 2.35 (IQR 0.91-23.1) vs. 0.24 (IQR 0.12-0.38) 10^4 RLU/min/20 million sperms) (Venkatesh et al., 2011).

In a case-control study, lipid peroxidation (2-thiobarbituric acid-reactive substances (TBARS) as substitute for MDA, arachidonic acid (AA), docosahexaenoic acid (DHA)) in semen was evaluated in 12 males with UI and 17 controls. TBARS and AA levels in blood plasma were found to be significantly higher in the UI males group compared to controls (Oborna et al., 2010).

In a case-control study, ROS in semen were compared between 54 men with UI and 51 fertile sperm donors. Significant higher ROS levels were found in semen of UI patients compared to controls (0.35 ± 0.67 vs. 0.01 ± 0.02 x10^6 cpm/20x10^6 sperm) (Desai et al., 2009).

In a case-control study, DNA damage and oxidative stress (total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI)) measured by Comet assay in semen of 30 males with UI and 20 fertile donors was investigated. No significant differences were found between UI and control males for TAO, TOS, OSI or sperm DNA damage (Verit et al., 2006).

In a case-control study, metabolomic analysis was performed on the urine of 71 UI and 47 fertile males The study was able to distinguish significant differences between the two groups with respect to a number of purported biomarkers (Zhang et al., 2014).

Female

In a prospective cohort study, oxidative stress markers were evaluated in the serum and follicular fluid of 31 women with UI and compared to 40 women with male factor infertility. Serum Fas, follicular fluid MDA and follicular fluid TAC levels were found to be significantly lower in women with UI compared to controls (sFAS 2.85±0.44 vs. 2.90±1.01 pg/ml; fMDA 3.19±0.21 vs. 3.47±0.30 µM and fTAC 0.88±0.16 vs. 1.31±0.63 mmol/L respectively). Serum and follicular fluid superoxide dismutase (SOD) levels were found to be significantly higher in women with UI compared to controls (3.59±0.45 vs. 3.47±0.68 U/mL and 4.66±1.64 vs. 3.14±0.91 U/mL) (Pekel et al., 2015).

In a case-control study, including 20 women with UI and 20 controls undergoing ICSI for male-factor infertility, the association between oxidant status (TOS, TAS, and OSI) and infertility and outcomes after ICSI was investigated. TOS and OSI in follicular fluid of the UI patients were statistically higher than the control group (10.14±6.69 vs. 6.54±3.52 µmol H2O2 Eq/L and 0.94±0.5 vs. 0.62±0.33 arbitrary units, respectively). The systemic TOS and OSI were also significantly increased in the UI group compared to the control group (9.63±6.16 vs. 5.51±4.27 µmol H2O2 Eq/L and 0.82±0.5 vs. 0.47±0.35 arbitrary units, respectively). No significant difference in implantation, clinical PR or LBR (Şentürk et al., 2021).

In a case-control study, including 145 infertile women with different infertility diagnosis and 35 controls, the association between follicular fluid metabolites and infertility was investigated. The concentrations of 27 metabolites in follicular fluid were found to be significantly different between infertile females and controls. The pattern of alterations in the aforementioned metabolites was different according to different infertility diagnoses (Lazzarino et al., 2021).

In a case-control study, oxidative stress (MDA, serum nitrite and FRAP) was evaluated in 13 women with UI and compared to 25 fertile controls. Serum nitrite was lower in UI compared to controls (3.0±0.43 vs. 5.0±0.52 µmol/L). Serum MDA levels were significantly higher in UI compared to controls (3.28±0.10 vs. 2.82±0.15 nmol/L) (Veena et al., 2008).

Couple

In a case-control study, the role of superoxide dismutase (SOD) 2 and nitric oxide synthase (NOS) polymorphisms in UI was evaluated. Sixty-nine fertile patients (34 men and 35 women) and 110 infertile patients with UI (52 men and 58 women) were enrolled. Comparing fertile and infertile groups, a significant difference was noted only for the eNOS gene. Homozygosity for the 894G-eNOS allele was associated with a significant increased risk of infertility (OR 1.91, 95% CI 1.04-3.54). For males with UI, the Ala-MnSOD allele was found in 59% of UI males vs. 41% of controls, and associated with infertility (OR 2.94, 95% CI 1.14-7.60) (Faure et al., 2014).

Recommendation

Measurement of oxidative stress in semen of males with unexplained infertility should only be considered in the context of research.	Research only	

Measurement of oxidative stress in women with unexplained infertility is not recommended.	Strong	⊕⊕○○

Justification

The included studies point towards an increase in ROS in males with UI. However, studies described have used many different methods, some of which are not readily available, limiting access and equity. The evidence for male testing is low and would benefit from further research. Patients would value a simple blood test if treatment was available and effective. In the absence of this, testing of oxidative stress should be standardised across research studies and treatment benefits compared with the work and expenses involved in testing. In females, there is good evidence that testing is not valuable, at least for serum, while follicular fluid is difficult to obtain before fertility treatment. Again, there is no evidence of any treatment being effective thereby limiting investigation and the cost of testing.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.10).

GENETIC/GENOMIC TESTS

Evidence

In a retrospective cohort study, 4345 (2261 male and 2084 female) individuals with reproductive disorders (11% of patients with UI) underwent karyotype testing. Abnormalities were found in 3% of patients with UI, compared with 2.2% for ART failure and 1.6% for recurrent miscarriage. No statistical analysis was performed (Ertosun et al., 2022).

In a prospective cohort study, genetic polymorphisms in the FSHB gene were evaluated in 36 females with UI and 169 healthy women without known fertility problems. Carriers of the FSHB-211 T-allele had significantly higher serum FSH and LH concentrations, and this allele was enriched among infertility patients, and even nearly doubled in women with UI (23.6% vs. 12.4%). Frequency of TT homozygosity was increased threefold (5.6% vs. 1.8%) (Rull et al., 2018).

In a prospective cohort study, 98 couples with UI were screened for genetic polymorphisms in the PPAR gamma gene. No relationship was found between pregnancy rate and the studied polymorphisms (Sahmani et al., 2011).

In a prospective cohort study, 19 women with UI were screened for genetic polymorphisms of CIAS1, an inflammasome component. Frequency was not significantly different between groups, 18.4% in unexplained vs. 17% in male infertility (Witkin et al., 2010).

In a case-control study, sperm miRNA expression levels were evaluated in 8 males with UI and 10 fertile controls. Overall, 115 miRNAs were found to be ubiquitous in all normospermic infertile individuals, while 59 miRNAs were not detected. In addition, 57 miRNAs were found to be differentially expressed; of which 20 are regulated by a host promoter and 3 of these comprised genes involved in fertility (Salas-Huetos et al., 2016).

In a case-control study, 28 normozoospermic men with female partners with UI were screened for chromosomal polymorphisms. Chromosomal polymorphisms were found in 9/28 normozoospermic men (Suganya et al., 2015).

In a case-control study, 206 males with UI and 230 healthy controls were screened for genetic polymorphisms of MTHFR. No significant differences in allele frequencies between males with UI and controls were found (Vani et al., 2012).

In a study, including 1206 normovulatory subfertile women, the association between chromosome abnormalities and infertility was investigated. The cause of infertility was not associated with the prevalence of chromosome abnormalities in the patients analysed. However, a significantly higher prevalence of chromosome abnormalities was observed in women with secondary infertility (1.25%) compared to those with primary infertility (0.25%) (Papanikolaou et al., 2005).

A study including 50 couples with UI investigated the association between chromosome abnormalities and infertility. Significantly more micronucleated cells were found in infertile couples compared to controls (14.66±5.21 vs. 10.60±2.57) (Trková et al., 2000).

Recommendation

Genetic or genomic tests are currently not recommended in couples with unexplained infertility.	Strong	⊕○○○

Justification

Many tests are described in literature, but there is little evidence of a specific benefit in UI, as opposed to the general infertility population. Genetic testing is expensive, and there is currently no association between specific genes and UI. While background rates of chromosomal analysis vary between studies, there is little evidence of an increase in UI in either men or women. Patients would value knowing their karyotype is normal, but a routine karyotype may not be sufficient to exclude genetic contributions to infertility. Selection of specific genes contributing to UI has not shown any benefit and the cost is large relative to the benefit. Assessment of any abnormality will need advice from a genetic specialist and expensive intervention via IVF and PGT-A, depending on the particular genetic condition.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.10).

VITAMIN D DEFICIENCY

Evidence

In a study including 58 men with UI and 50 age- and BMI-matched fertile men, vitamin D levels were compared between groups. Compared with the fertile group, male patients in couples with UI had significantly lower vitamin D levels (27.00 ng/mL (12.63-39.30) vs.23.66 ng/mL (7.50-55.00)) (Güngör et al., 2022).

A retrospective cohort study, including women undergoing their first IVF cycle, investigated the association between vitamin D and live birth rates. The cumulative live birth rate in the vitamin D-deficient group was significantly lower compared to the non-deficient group (43.9%, 208/474 vs. 50.9%, 325/639, OR 0.755, 95% CI 0.595–0.959). The clinical/ongoing pregnancy rate, live birth rate and miscarriage rate in the fresh cycle did not show significant differences between the vitamin D deficient and non-deficient groups (Ko et al., 2022).

In a sub-analysis of a randomized controlled trial, the association between vitamin D deficiency and UI was investigated in 647 women. Overall, 25% of patients met the criteria for vitamin D deficiency. In patients with vitamin D deficiency the live birth rate was not significantly different from those who were not vitamin D deficient (32 vs. 29%, OR 1.1, 95% CI 0.7-1.7) (Butts et al., 2019).

In a retrospective cohort study, the influence of vitamin D levels on IVF outcomes was investigated in 22 women with UI. Overall, 14/22 women had vitamin D levels >30 ng/ml, 4 had levels 20-30 ng and 4 had levels <20 ng/ml. There was no specific effect on UI but vitamin D deficiency was associated with lower pregnancy rates in non-Hispanic white females but not in Asian females (Rudick et al., 2012).

In a case-control study, the prevalence of vitamin D deficiency was evaluated in 26 women with UI and compared to 15 women with male factor infertility. Vitamin D levels were not significantly different between women with UI and women with male factor infertility (23.3 ± 8.6 vs. 26.2 ± 9.2 ng/ml) (Lopes et al., 2017).

Recommendation

Testing for vitamin D deficiency is not recommended for diagnosis of unexplained infertility.	Strong	⊕OOO

Justification

In the context of UI, no role of vitamin D has been found. The evidence is of relatively low quality, but generally against specific testing outside of other medical or environmental indications. Testing for an individual is relatively inexpensive and widely available with easy dietary remediation, but treatment efficacy is unproven.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.10).

PROLACTIN

Evidence

In a cross-sectional study, the association between prolactin and UI was investigated. 84 women with UI and 44 healthy fertile women were enrolled and ROC curves were calculated. Prolactin levels were significantly higher (2-fold elevated) in serum of women with UI compared to controls. Using prolactin, MCP-1 and leptin in a predictive model, a significant receiver operating curve (ROC of 0.89) was obtained for prediction of UI (Qu et al., 2020).

In a prospective cohort study, the role of prolactin in UI was investigated in 12 women with UI and 12 fertile controls. Midcycle bioactive prolactin (34.2±8.3 vs. 19.2±3.4 ng/ml) but not immunoactive (26.9±4.3 vs. 22.1±2.6 ng/mL) were different in women with UI compared to controls (Subramanian et al., 1997).

In a cross-sectional study, the association between prolactin and UI was investigated in 187 women with UI and compared with 52 women with male infertility. Prolactin levels were not significantly different in the UI group compared to the male factor infertility group (10.4 ng/mL (IQR 7.7-13.4) vs. 11 ng/mL (IQR 8.5-13.7) (Orouji Jokar et al., 2018).

In a case-control study, prolactin levels were no different in 13 women with UI compared to 25 fertile controls. Lactate dehydrogenase (LDH) was also evaluated and were significantly higher in serum in UI compared to controls (83.40±4.81 vs. 67.9±3.53 U/L) (Veena et al., 2008).

Recommendation

Prolactin testing in women is not recommended.	Strong	⊕OOO

Justification

The included studies are small and the quality of the data is very poor and heterogenous. In addition, current evidence is unable to show a benefit to measuring prolactin levels in asymptomatic women with UI. While the testing is cheap and widely available, the evidence is low and the cost benefit relationship poor.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.10).

BMI

Evidence

In an epidemiologic survey study, the association of dietary status and UI was evaluated in 198 women with UI and 59 pregnant controls. There was a significant difference in caloric intake between women with UI and controls (2688.64±580.78 vs. 2115.44±326.63 calories), with a lower intake of carbohydrates and vitamins and higher intake of fats in women with UI. Of the women with UI 33% reported daily physical exercise compared to 69% of controls (Noventa et al., 2016).

In an epidemiologic survey study, the effect of body weight on the success rate of IVF in 1828 women with UI was studied. A significantly higher live birth rate per cycle was found in women with normal weight (BMI ≥20–25 kg/m^2) and slight overweight (BMI 25–27 kg/m^2) compared with women with a BMI ≥27 kg/m^2. The unfavourable effect of overweight was largest for women with UI (Lintsen et al., 2005).

In a study, the effect of BMI on estradiol, progesterone and LH values in females who received IUI treatment was analysed in women with UI. Estradiol on the day of hCG was lower in overweight/obese (natural and stimulated cycles) where patients were <35 years but not in over 35 years. In older women estradiol, progesterone and LH were lower in woman with greater weight (Wang et al., 2020).

Recommendation

BMI evaluation in women is considered good practice in pre-conception care.	GPP

Justification

Although there is little evidence of a specific association between BMI and UI specifically, reproductive outcomes are known to be impaired in men and women with low and high BMIs. The standard advice and medical investigation and interventions apply equally to patients with UI as to any other causes of infertility. Patients generally value advice about lifestyle and healthy alternatives to maximise fertility in the context of their social and cultural environment. While healthy lifestyle intervention may improve spontaneous conception, active weight loss treatment in assisted reproduction has not yet shown a benefit in getting pregnant.

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question II.10).

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III. Treatment

III.0 When to start treatment

It is known that couples presenting with Unexplained Infertility (UI) can achieve spontaneous pregnancy. Models have been developed to predict the chance of spontaneous pregnancy, such as the Hunault model (Hunault et al., 2004). Typically, such models use a validated set of prognostic factors shown to impact the chance of spontaneous pregnancy and consider the weight or importance of the prognostic factors. The most important prognostic factors are age, duration of infertility, previous treatment and previous pregnancy.

It is however important to note that none of the currently available prediction models are fully evolved. The downside of the Hunault model for example, is that it has only been validated for the Canadian and the Dutch population, and more importantly, that because of the way it has been developed, it can only be used once: at the point of diagnosis, when couples first present with infertility.

More evolved dynamic models which can be used more than once to provide updated estimates of chances of natural conception over 6 -12 months have been developed (McLernon et al., 2019, van Eekelen et al., 2017). Although these have undergone initial validation in Dutch and Scottish populations, none are in common use as they have yet to be validated using data from other settings or implemented in clinical practice.

As shown in the next two chapters, the perception that a treatment is either effective or not effective does not apply to UI. Namely, one RCT comparing ovarian stimulation and IUI to expectant management in good-prognosis patients found no difference in live birth rate (Steures et al., 2006), while another RCT investigating the same treatment comparison in poor-prognosis patients reported a striking benefit of treatment (ovarian stimulation and IUI) over expectant management (Farquhar et al., 2018). Therefore, young women with a short duration of infertility have a high prognostic index and the added benefit of active treatment is small. However, with longer duration of infertility and older age, the prognostic index decreases and the benefit of active treatment increases.

Prognostic models can help the decision-making on a treatment plan in couples with unexplained infertility, however, it is also important to take patient preferences into account when deciding on treatment options.

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III.1 Expectant management

PICO QUESTION: WHAT IS THE VALUE OF EXPECTANT MANAGEMENT COMPARED TO ACTIVE TREATMENT FOR PATIENTS WITH UI?

For this chapter, the GDG considered timed intercourse without hormonal stimulation part of expectant management and not as an active treatment, hence the comparison of timed intercourse without hormonal stimulation versus expectant management is not included in the guideline.

CLOMIPHENE CITRATE WITH TIMED INTERCOURSE (+/- OVULATION TRIGGER) VS. EXPECTANT MANAGEMENT

Evidence

One RCT including 385 patients with unexplained infertility (UI), compared six months of expectant management (n=167) with clomiphene citrate (CC) and timed intercourse (n=173). Cumulative birth rate was 16% (26/167) with expectant management compared to 13% (23/173) with active treatment. Compared with expectant management, the adjusted hazard ratio (HR) for the time to a pregnancy leading to a live birth was 0.83 (99% CI 0.42-1.63) (Bhattacharya et al., 2008). The cost-benefit study using this data by the same group also found no cost-benefit of clomiphene citrate over expectant management (Wordsworth et al., 2011).

In a four-arm RCT, including 155 couples with UI, timed intercourse with CC for ovarian stimulation (OS), with or without hCG for final oocyte maturation, was compared to timed intercourse and placebo, with or without hCG for final oocyte maturation. Pregnancy rates were significantly higher after timed intercourse with CC and hCG compared to placebo without hCG (7/37 (19%) vs. 0/36 (0%) (Fisch et al., 1989).

LETROZOLE WITH TIMED INTERCOURSE (+/- OVULATION TRIGGER) VS. EXPECTANT MANAGEMENT

Evidence

No relevant studies were identified comparing letrozole with timed intercourse (+/- ovulation trigger) with expectant management in couples with UI.

GONADOTROPINS WITH TIMED INTERCOURSE (+/- OVULATION TRIGGER) VS. EXPECTANT MANAGEMENT

Evidence

No relevant studies were identified comparing OS with gonadotropins and timed intercourse (+/- ovulation trigger) with expectant management in couples with UI.

INTRA-UTERINE INSEMINATION (IUI) IN A NATURAL CYCLE VS. EXPECTANT MANAGEMENT

Evidence

One RCT compared IUI in a natural cycle with expectant management in couples with UI. Live birth rate was not significantly different between IUI and expectant management (38/165 (23%) vs. 26/167 (16%) (Bhattacharya et al., 2008).

Ovarian stimulation with IUI vs. expectant management

Evidence

A systematic review and meta-analysis compared OS combined with IUI and expectant management in couples with UI. The OR for cumulative live birth rate in couples with poor prognosis was 4.48 (95% CI 2.00-10.01, 1 RCT, 201 women). The OR for live birth rate in couples with moderate prognosis was 0.82 (95% CI 0.45-1.49; 1 RCT, 253 women). The OR for multiple pregnancy rate was 3.01 (95% CI 0.47-19.28; 2 RCTs, 454 women) (Ayeleke et al., 2020).

IVF vs. expectant management

Evidence

A systematic review and meta-analysis compared IVF with expectant management. The OR for live birth with 1 cycle of IVF compared to three months of expectant management was 22.0 (95% CI 2.56-189.38, 51 women, 1 RCT). The OR for clinical pregnancy with 1 cycle of IVF compared to 3-6 months of expectant management was 3.24 (95% CI 1.07-9.80, 2 RCTs, 86 women). Although the evidence is of low quality and insufficient, IVF is presently associated with a higher live birth rate than expectant management (Pandian et al., 2015).

In a retrospective cohort study, 635 couples with UI and female age ≥39 years were included. Couples undergoing immediate IVF treatment (n= 359) were compared to couples waiting for about 1 year to start IVF treatment (n=276). No significant difference was found in live birth rate between immediate IVF treatment and waiting for about a year (70/359 (19%, 11 natural conception and 59 after IVF) vs. 57/276 (20.7%, 37 natural conception and 20 after IVF) (Carosso et al., 2022).

Overall recommendation

Recommendation

IUI with ovarian stimulation is recommended as a first-line treatment for couples with unexplained infertility.	Strong	⊕○○○

The GDG advices to base the decision to start active treatment on prognosis in couples with unexplained infertility.	GPP

Justification

The first mentioned RCT (Bhattacharya et al., 2008) is by far the largest and latest and shows no significant evidence that CC is either more efficient or cheaper than expectant treatment. Similar findings were reported for IUI in a natural cycle versus expectant management. Although only one RCT is available, it is of sufficient quality and size to suggest that the live birth rate following IUI in a natural cycle is not significantly superior to that following expectant management.

The weight of evidence strongly suggests that IUI with OS is recommended in preference to expectant management, particularly for couples with poor prognosis. Although IUI involves obviously more invasive treatment, the difference in live birth rates between these two alternatives provides justification for its use. The latest (mainly European) studies suggest that only a low-dose regimen should be employed when using gonadotrophins for OS, since it can greatly reduce the multiple pregnancy rate without significantly reducing the live birth rate.

For the comparison of IVF to expectant management, the evidence is scarce. The current evidence seems to point towards a higher efficacy for IVF than expectant management, leading to the conclusion that IVF is recommended over expectant management. The GDG regards this is as indirect evidence for the effectiveness of IVF and regards it unlikely that direct evidence from trials comparing IVF vs. expectant management will emerge in the future due to several factors. These include whether equipoise exists for such a trial is controversial, secondly the proper comparator of IVF is unclear, i.e., how many cycles of expectant management. Also, IVF comes with high physical and psychological burdens. In the absence of direct evidence for effectiveness and or additional benefit of IVF in different patient profiles, the GDG opinion is that the decision to use IVF should be based on patient characteristics and preferences.

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.1).

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III.2 Active treatment

PICO QUESTION: IF ACTIVE TREATMENT IS PURSUED, WHICH TYPE OF ACTIVE TREATMENT FOR UI?

TIMED INTERCOURSE

Clomiphene citrate and timed intercourse vs. letrozole and timed intercourse

Evidence

In a RCT, including 172 women with unexplained infertility (UI), timed intercourse with CC for ovarian stimulation (OS) was compared to letrozole. Comparing CC to letrozole, no significant differences were noted in ongoing pregnancy rate (7/86 (8.1%) vs. 11/86 (12.7%)) or multiple pregnancy rate (2/86 (2.3%) vs. 0/86 (0%)) (Harira, 2018).

In another RCT, including 270 women with UI, timed intercourse with CC for OS was compared to letrozole. Clinical pregnancy rate was significantly higher in the letrozole group (23.07 vs. 10.68%). Also, multiple pregnancy rate was significantly higher in the CC group (21.42 vs. 3.33 %) (Ibrahim et al., 2012).

Gonadotropins and timed intercourse vs. clomiphene citrate or letrozole and timed intercourse

Evidence

No relevant studies were identified comparing timed intercourse with letrozole to OS and IUI in couples with UI.

TIMED INTERCOURSE VS. IUI IN A NATURAL CYCLE

Clomiphene citrate and timed intercourse vs. IUI in a natural cycle

Evidence

In a 3-arm RCT, expectant management was compared with CC and timed intercourse and IUI in a natural cycle. Compared to CC with timed intercourse, treatment with IUI resulted in a higher live birth rate (13% (23/173) vs. 23% (38/165)) (Bhattacharya et al., 2008). However, the RCT was not powered to compare the two active treatment arms to each other.

Letrozole and timed intercourse vs. IUI in a natural cycle

Evidence

No relevant studies were identified comparing timed intercourse with letrozole to IUI in a natural cycle in couples with UI.

Gonadotropins and timed intercourse vs. IUI in a natural cycle

Evidence

No relevant studies were identified comparing timed intercourse with gonadotropins to IUI in a natural cycle in couples with UI.

TIMED INTERCOURSE VS. OVARIAN STIMULATION AND IUI

Clomiphene and timed intercourse vs. ovarian stimulation and IUI

Evidence

In a RCT, including 113 couples with UI, couples were randomised to either receive CC and timed intercourse or CC and IUI. Conception rates were not statistically different between timed intercourse and IUI (28/69 (41%) vs. 8/44 (18%)) (Agarwal and Mittal, 2004).

Letrozole and timed intercourse vs. ovarian stimulation and IUI

Evidence

No relevant studies were identified comparing timed intercourse with letrozole to OS and IUI in couples with UI.

Gonadotropins and timed intercourse vs. ovarian stimulation and IUI

Evidence

A systematic review and meta-analysis, including two RCTs, compared OS with timed intercourse to OS and IUI. It is uncertain whether OS and IUI results in a higher live birth rate than OS with gonadotropins and timed intercourse (OR 1.59, 95% CI 0.88-2.88, 2 RCT, 208 women). It is uncertain whether OS and IUI results in a lower multiple pregnancy rate (OR 1.61, 95% CI 0.44-5.89, 2 RCT, 208 women) than OS with gonadotropins and timed intercourse (Ayeleke et al., 2020).

IUI IN A NATURAL CYCLE VS. OVARIAN STIMULATION AND IUI

Evidence

A systematic review and meta-analysis, including four RCTs, compared OS with IUI to IUI in a natural cycle. Live birth rate was higher with OS and IUI compared to IUI in a natural cycle (OR 2.07, 95% CI 1.22-3.50, 4 RCT, 396 women). It is uncertain whether OS and IUI result in a higher multiple pregnancy rate (OR 3.00 95% CI 0.11-78.27, 1 RCT, 39 women) (Ayeleke et al., 2020).

IUI vs. IVF

Evidence

A systematic review and meta-analysis compared IVF with IUI in a natural cycle. Live birth rate was higher with IVF compared to unstimulated IUI (OR 2.47, 95% CI 1.19-5.12, 2 RCT, 156 women). There was no evidence of a difference in multiple pregnancy rate (OR 1.03, 95% CI 0.04-27.29, 1 RCT, 44 women) (Pandian et al., 2015).

Another systematic review and meta-analysis, including 8 RCTs with 1497 couples with UI, compared efficacy and safety of IVF and IUI with OS (Nandi et al., 2022). Live birth rate was significantly higher after IVF compared to IUI with OS (RR 1.54, 95% CI 1.04-2.28, 7 RCT, 1391 women). No significant difference between groups was found for multiple pregnancy rate (RR 0.83, 95% CI 0.50-1.38, 6 RCT, 507 women) or OHSS (RR 1.77, 95% CI 0.49-6.37, 3 RCT, 981 women). In a sensitivity analysis including only studies with women without previous treatment, no significant difference in live birth rate was found in women <38 years (RR 1.01, 95% CI 0.88-1.15, 3 RCT, 925 women). However, in women ≥38 years, live birth rate was significantly higher after IVF treatment (RR 2.15, 95% CI 1.16-4.00, 1 RCT, 154 women) (Goldman et al., 2014, Nandi et al., 2022).

Overall recommendation

Recommendation

IUI with ovarian stimulation is recommended as a first-line treatment for couples with unexplained infertility	Strong	⊕○○○

To avoid multiple pregnancies and OHSS, care is needed by using gonadotrophin treatment only in a low-dose regimen with adequate monitoring.	GPP

IVF is probably not recommended over IUI with ovarian stimulation in couples with unexplained infertility.	Conditional	⊕○○○

It is expected that the decision to use IVF is individualized by patient characteristics such as age, duration of infertility, previous treatment and previous pregnancy.	GPP

Justification

There is a lack of high-quality RCTs on the topic. Clomiphene, letrozole, gonadotrophins and IUI on their own are not effective compared to expectant management or IUI in a natural cycle. On the other hand, IUI in combination with OS is not inferior to IVF. Furthermore, the additional costs and risks of IVF need to be considered. Therefore, IUI in a stimulated cycle for three to six cycles is viewed as the first-line treatment in couples with unexplained infertility. This is taking into account low-dose gonadotropins for OS to avoid a high number of growing follicles, which can increase the risk of multiple pregnancies.

The decision to use IVF is individualized by patient characteristics, such as age, duration of infertility, previous treatments and previous pregnancy. Current evidence shows that in treatment-naïve patients, IVF is as effective as IUI with OS. However, as the invasiveness of the procedure and the costs are considerably lower with IUI, the GDG concluded that OS and IUI is recommended as the first-line treatment.

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.2).

PICO QUESTION: WHAT IS THE VALUE OF IVF VERSUS ICSI?

Evidence

An RCT, including 60 couples with UI, compared IVF with ICSI. There were no differences in live birth rate between the IVF and ICSI groups (14/30 (46.7%) vs. 15/30 (50%)) (Foong et al., 2006).

A subgroup analysis of a large RCT included 382 couples with UI, randomly assigned to IVF (n=183) and ICSI (n=199), found no significant difference in live birth rate between groups (35.5% (65/183) vs. 36.7% (73/199), RR 1.03 (95% CI 0.79-1.35)) (Dang et al., 2021).

A subgroup analysis of an RCT included 100 couples with UI. There was no difference in pregnancy rates between IVF and ICSI (32% vs. 38%, RR 0.83, 95% CI 0.48-1.45) (Bhattacharya et al., 2001).

Recommendation

ICSI is not recommended over conventional IVF in couples with unexplained infertility.	Strong	⊕OOO

Justification

Evidence from RCTs comparing IVF with ICSI in coupes with UI showed comparable live birth rates. Furthermore, there is substantial evidence from RCTs showing no difference in live birth rate between IVF and ICSI for non-male factor infertility (Bosch et al., 2020, Dang et al., 2021). Given this overriding evidence, and the additional resources and costs associated, ICSI is not routinely recommended for UI.

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.2).

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III.3 Mechanical-surgical procedures

PICO Question: What is the value of mechanical-surgical procedures?

Resection of polyps or fibroids

Evidence

Spontaneous conception

In an RCT, 200 women with at least two years of unexplained infertility (UI) and without suspicion of uterine abnormalities were randomly assigned to have office microhysteroscopy or not. These couples were trying to conceive spontaneously. In the microhysteroscopy group, when pathology was detected, treatment was done, including hysteroscopic resection of endometrial polyps and submucous fibroids, and excision of a uterine septum. In 70% of patients no uterine abnormality was found. The primary outcome of live birth was not reported. In favour of microhysteroscopy the ongoing PR was 43/100 vs. 10/100 (RR 4.30, 95% CI 2.29-8.07), clinical PR was 57/100 vs. 15/100 (RR 3.80, 95% CI 2.31-6.24). No adverse events were reported (Seyam et al., 2015).

In a RCT, 94 women with UI (≥1 year of fertility-oriented intercourse, normal ovarian function, normal hysterosalpingography, a normal post-coital test and normal semen analysis of the partner) but with fibroids on transvaginal ultrasound, were assigned to have the fibroids removed or not. If there was an intramural fibroid <4.0 cm this was removed by laparotomy and in case of a submucous fibroid this was removed hysteroscopically. It is uncertain if surgical removal of fibroids improved clinical pregnancy rates (OR 2.44, 95% CI 0.97-6.17). There was insufficient evidence to show a benefit of fibroid removal on miscarriage rates (OR 1.54, 95% CI 0.47-5.00) (Casini et al., 2006).

Recommendation

Hysteroscopy for the detection and possible correction of intrauterine abnormalities not seen at routine imaging is not recommended.	Strong	⊕⊕OO

Justification

There is insufficient evidence on the effectiveness of hysteroscopic surgery in women with unexplained infertility. The current evidence does not support the correction of these minor intrauterine abnormalities Reported randomised trials are at serious risk of bias and other studies lack control groups. In the setting of first IVF cycles, no significant difference in live birth rate was found (RR 1.20; 95% CI 0.96-1.49, 2 RCT, 953 women) (Kamath et al., 2019). Surgery is only advised in a well-controlled research setting (preferably randomised trials) in women with well-defined definitions for abnormality and clinical outcome.

For treatment of uterine fibroids in women with otherwise unexplained infertility there is insufficient evidence to make any recommendation.

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.3).

TUBAL FLUSHING

Evidence

A systematic review and meta-analysis, including 15 RCTs and 3864 women with subfertility, investigated the effect of tubal flushing with oil-soluble contrast media (OSCM) or water-soluble contrast media (WSCM) on reproductive outcomes (Wang et al., 2020).

Oil soluble contrast media (OSCM) versus no flushing
OSCM may increase the odds of live birth (OR 3.27, 95% CI 1.57-6.85, 3 RCTs, 204 women) and clinical pregnancy (OR 3.54, 95% CI 2.08-6.02, 4 RCTs, 506 women) (Wang et al., 2020).

Water soluble contrast media (WSCM) versus no flushing
It is uncertain whether flushing with WSCM increases live birth rate (OR 1.13, 95% CI 0.67-1.91, 1 RCT, 334 women). It is uncertain whether flushing with WSCM increases clinical pregnancy rate (OR 1.14, 95% CI 0.71-1.84, 1 RCT, 334 women) (Wang et al., 2020).

Oil versus water soluble contrast media
Live birth rate was reported in 3 RCTs. In two RCTs, a higher live birth rate was reported with OSCM (OR 1.64 95% CI 1.27-2.11, 1119 women; OR 3.45, 95% CI 1.97-6.03, 398 women). In one RCT, no evidence of a difference between groups was found (OR 0.92, 95% CI 0.60-1.40, 533 women). Tubal flushing with OSCM probably increases the odds of clinical pregnancy compared to WSCM (OR 1.42, 95% CI 1.10-1.85, 6 RCTs, 2598 women). Flushing with OSCM probably increased the odds in intravasation (OR 5.00, 95% CI 2.25-11.12, 4 RCTs, 1912 women). No difference in infection or haemorrhage between OSCM and WSCM and no serious adverse events reported (Wang et al., 2020).

The largest trial comparing oil versus water soluble contrast media involved 1119 (OSCM: n=557 vs. WSCM: n=562) infertile women undergoing HSG with a 5 year follow-up . In the OSCM group, 39.8% of the women needed no other treatment, 34.6% underwent IUI and 25.6% had IVF/ICSI in the 5 years following HSG. In the WSCM group, 35.0% of the women had no other treatment, 34.2% had IUI and 30.8% had IVF/ICSI in the 5 years following HSG (p=0.113). During the 5-year period there was a significantly higher ongoing pregnancy rate (RR 1.07, 95% CI 1.00-1.14) and live birth rate (RR 1.11, 95% CI 1.03-1.20) and a shorter time to ongoing pregnancy (10.0 months (95% CI 8.5-11.5) vs. 13.7 months (95% CI 11.7-15.8)), in favour of OSCM compared with WSCM used at HSG (van Welie et al., 2021).

Recommendation

HSG (i.e., tubal flushing) with an oil-soluble contrast medium is preferable over a water-soluble contrast medium. Risks and benefits of tubal flushing with oil-soluble contrast medium should be discussed with all couples with unexplained infertility.	Conditional ⊕⊕○○

Justification

Current evidence shows that HSG performed with OSCM is preferred over WSCM. This may lead to more clinical pregnancies and live births at no extra cost. Part of the evidence for this question was derived from ovulatory infertile women with diagnosis other than UI. However, the largest trial by Dreyer et al., 2017, included 1119 women with UI (Dreyer et al., 2017).

Risks of tubal flushing with oil-based contrast are low with the most frequently reported complication being intravasation. Since the introduction of oil-based contrast in 1928 serious consequences of embolism have been reported in four cases, emphasising the importance of performing HSG's under fluorescence guidance in order to abandon the procedure in a timely manner (Roest et al., 2021).

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.3).

Minimal to mild endometriosis

Evidence

In a Cochrane systematic review evaluating the role of laparoscopic surgery versus diagnostic laparoscopy in women with minimal or mild endometriosis, results from 3 randomised trials were pooled (Bafort et al., 2020). There was an improvement in clinical pregnancy rate with laparoscopy (OR 1.89, 95% CI 1.25-2.86, 3 RCTs, 528 participants). There was insufficient data on the safety of the intervention.

Conclusion

If incidentally minimal to mild endometriosis is found at laparoscopy, this is not further considered unexplained infertility by the GDG.

Justification

The GDG recommends against routine laparoscopy. If incidentally minimal to mild endometriosis is found, this is considered outside the scope of this guideline. The reader is referred to the ESHRE guideline on Endometriosis (Becker et al., 2022).

Further information

Details of the literature study and evidence tables are available in Annex 6 and Annex 7 (question III.3).

ENDOMETRIAL INJURY/SCRATCHING

Evidence

Timed intercourse

A recent randomised trial in women with UI included 220 women who were randomized. Endometrial biopsy or placebo procedure took place between D1-12 of the menstrual cycle; second attempt was allowed if the first was unsuccessful. Couples had regular unprotected sexual intercourse for 3 cycles. There was no difference in the outcomes of live birth (10/113 (9%) vs. 7/107 (7%); OR 1.39, 95% CI 0.50-4.03), ongoing pregnancy (10/113 (9%) vs. 7/107 (7%); OR 1.39, 95% CI 0.50-4.03) or miscarriage (2/113 (2%) vs. 1/107 (1%); OR 20.01, 95% CI 0.19-43.82) (Wong et al., 2022).

An RCT with 234 women with UI undergoing timed intercourse with OS, randomised women to receive endometrial biopsy on the day of LH surge detection or mock biopsy. Ongoing pregnancy rate was significantly higher in women receiving endometrial scratch (17/114 (14.9%) vs. 6/103 (5.8%); OR: 2.83 95% CI 1.07-7.49)). There was no significant difference in miscarriage rate (3 (17.64%) vs. 1 (14.28%)) (Parsanezhad et al., 2013).

IUI

In an RCT with 96 women suffering from infertility of unknown cause undergoing IUI with OS, women received an endometrial scratch in the midluteal phase (days 21–26 of the cycle, n=54) or no endometrial injury (n=42). There was no statistical difference in ongoing pregnancy rate (4/54 (10%) vs. 2/42 (4.76%)) between women undergoing endometrial scratch or not. Multiple pregnancies and miscarriages were not observed (Yildiz et al., 2021).

In a randomised trial, 150 women with UI and an indication for IUI were randomised between scratch on day 6-7 of their treatment cycle or no scratch. Couples received up to 3 cycles of IUI. There was no difference in ongoing pregnancy rate (6/75 (8.0%) vs. 8/75 (10.7%); RR 0.75, 95% CI 0.27-2.06) or multiple pregnancy rate (0/75 vs. 1/75) (Ghuman et al., 2020).

An RCT with 120 women suffering from infertility of unknown cause undergoing IUI with OS, randomised women to receive an endometrial injury on cycle day 3 or no endometrial injury. There were no significant differences in clinical pregnancy rate (11/59 (18.6%) vs. 10/59 (16.9%)) or miscarriage rate (1/59 (1.7%) vs. 3/59 (5.1%)) between women undergoing endometrial injury or not (Jafarabadi et al., 2020).

An RCT with 80 women suffering from infertility of unknown cause undergoing IUI with OS, randomised women to receive endometrial scratch in the midluteal phase (days 21–25 of the cycle) or no endometrial injury. There were no significant differences in clinical pregnancy rate (11/40 (27.5%) vs. 5/40 (12.5%)) between women undergoing endometrial scratch or not (Senocak et al., 2017).

An RCT with 154 women with UI, randomised patients to undergo OS and IUI with or without endometrial scratch on the day of final oocyte maturation. Clinical pregnancy rate was significantly higher in women receiving endometrial injury compared to controls (30/77 (39%) vs. 14/77 (18.2%)). There was no statistical difference in multiple pregnancy rate (2/77 (6.7%) vs. 1/77 (7.1%)) or abortion rate (5/30 (16.7%) vs. 3/14 (21.4%)) (Maged et al., 2016).

Recommendation

Endometrial scratching should not be offered for unexplained infertility.	Strong	⊕⊕OO

Justification

In addition to established therapies for UI such as IUI or IVF, endometrial scratching or injury has been proposed to increase the probability of pregnancy. It is hypothesised that local mechanical endometrial injury may enhance the receptivity of the endometrium and facilitate embryo implantation through inflammatory and immunological responses (Dekel et al., 2010, Gnainsky et al., 2015, Gnainsky et al., 2010, Granot et al., 2012).

Timing of injury of the endometrium differed in the studies. In some studies, scratching was performed in the luteal phase of the cycle preceding the treatment cycle (Senocak et al., 2017, Yildiz et al., 2021) in others, in the proliferative phase of the treatment cycle (Ghuman et al., 2020, Jafarabadi et al., 2020, Maged et al., 2016, Parsanezhad et al., 2013, Wong et al., 2022). In most studies, the endometrium was injured by taking a biopsy but in two an embryo transfer or biopsy catheter was used and moved up and down to injure the endometrium (Maged et al., 2016, Yildiz et al., 2021).

Although it is a low-cost procedure, which can be done at the outpatient clinic without anaesthetics, the evidence does not show better pregnancy outcome if scratching was performed before intercourse or before IUI in couples with UI.

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.3).

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III.4 Alternative therapeutic approaches

PICO QUESTION: WHAT IS THE EFFECTIVENESS OF ALTERNATIVE THERAPEUTIC APPROACHES?

ANTIOXIDANTS

Evidence

A systematic review and meta-analysis compared oral antioxidant treatment combined with an infertility treatment with placebo and infertility treatment in couples with unexplained infertility (UI). Similar live birth rates were reported in both groups (OR 1.50, 95% CI 0.60-3.72, 2 RCTs, 133 women). Multiple pregnancy rate was not significantly different (8.9% vs. 11.1%, OR 0.65, 95% CI 0.26-1.62, 1 RCT, 804 women) (Showell et al., 2020).

Recommendation

Adjunct oral antioxidant therapy to women undergoing fertility treatment is probably not recommended.	Conditional	⊕OOO

Adjunct oral antioxidant therapy to males undergoing fertility treatment is probably not recommended.	Conditional	⊕OOO

Justification

Current low-quality evidence does not show a benefit of antioxidant treatment in males or females with UI. Generally, antioxidants are not very expensive, however, their benefit was not demonstrated.

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.4).

ACUPUNCTURE

Evidence

One RCT including 76 women with UI investigated the effect of acupuncture sessions before and after embryo transfer (n=38) during IVF. Live birth rate was significantly higher in the acupuncture group vs. controls (52.8% (19/36) vs. 27.8% (10/36)). The STAI-1 score, indicative of anxiety levels, was significantly lower after embryo transfer in the acupuncture group vs. controls (28.8 (3.3) vs. 41.1 (6.8)) (Guven et al., 2020).

Recommendation

Acupuncture in women is probably not recommended	Conditional ⊕⊕OO

Justification

The evidence on acupuncture in unexplained infertility is very limited and of low quality. The selected study only looked at acupuncture in the context of IVF. Furthermore, in infertility, there is no agreement on the techniques of acupuncture, i.e., acupuncture points to use or timing. Therefore, acupuncture cannot be recommended for patients with unexplained infertility.

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.4).

NUTRACEUTICALS (INOSITOL)

Evidence

One RCT including 86 women with UI compared adjuvant treatment with myo-inositol suppositories (n=43) during timed intercourse cycles with placebo (n=43). Pregnancy rates were 18.6% (8/43) in the myo-inositol group compared to 6.97% (3/43) in the control group. No test of statistical significance was performed (Montanino Oliva et al., 2020).

Recommendation

Inositol supplementation in women is probably not recommended.	Conditional ⊕OOO

Justification

There is a plethora of nutraceuticals and the GDG was unable to find convincing evidence of benefit. The evidence quality was judged to be very low. Nutraceuticals are generally not very expensive, however, their benefit was not demonstrated.

Further information

Details of the literature study and evidence tables are available in Annex 6, Annex 7 and Annex 8 (question III.4).

PSYCHOTHERAPY

Evidence

No relevant studies were identified investigating the effect of psychotherapy in couples with unexplained infertility.

Recommendation

Psychological support, including psychotherapy, is recommended for patients when needed.	GPP

Justification

No studies were identified regarding psychotherapy in patients with UI specifically. However, psychotherapy can help infertility patients to improve health-related quality of life, anxiety and/or depression. Further information on psychosocial needs that patients experience across their treatment pathway, and how fertility clinic staff can detect and address these needs can be found in the ESHRE guideline on Routine Psychosocial Care (Gameiro et al., 2015).

TRADITIONAL CHINESE MEDICINE (TCM)

Evidence

Most literature was in Chinese language, where only publications in English were considered for this guideline.

One uncontrolled study was identified, where Onkyeong-tang and herbal medicine for ovulation and implantation were administered to women with UI. After treatment, the women were followed for 3 menstrual cycles of observation. Live birth rate was 7.8% (7/90), ongoing pregnancy per clinical pregnancy was 53.85% (7/13) and 37% (33/90) of women experienced adverse events, but none were serious (Choi et al., 2021).

DIET, EXERCISE, BEHAVIOURAL THERAPY

Behavioural therapy is an umbrella term for types of therapy that treat mental health disorders. This form of therapy looks to identify and help change potentially self-destructive or unhealthy behaviours. It is based on the idea that all behaviours are learned and that behaviours can be changed (McKay and Tryon, 2002).

Evidence

No relevant studies were identified investigating the effect of diet, exercise or behavioural therapy in couples with UI.

Recommendation

A healthy diet and regular exercise, supported by behavioural therapy when necessary, are recommended.	GPP

Justification

Although the GDG was unable to identify evidence of a specific association between certain diets or exercise regimes and unexplained infertility specifically, reproductive outcomes are known to be impaired in men and women with low and high BMIs. The standard advice and medical investigation and interventions apply equally to patients with UI as to any other causes of infertility. Patients generally value advice about lifestyle and healthy alternatives to maximise fertility in the context of their social and cultural environment. While healthy lifestyle intervention may improve spontaneous conception, active weight loss treatment in assisted reproduction has not yet shown a benefit in getting pregnant.

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IV. Quality of Life

PICO Question: Is there a difference in QoL for patients with unexplained versus explained infertility?

Evidence

In a secondary analysis of two RCTs, the fertility-related quality of life (QoL) was compared between couples with a known infertility cause (PCOS) and couples with unexplained infertility. The fertility-related quality of life was measured with FertiQoL; a higher score indicates a better QoL. With exception of the relational domain, women with PCOS (n=733) had lower FertiQoL scores than women with UI (n=865), which were largely explained by the differences in BMI, demographics and hirsutism between the groups. Males from a couple with UI (n=849) had lower scores than males partnered with women who had PCOS (n=641) (Santoro et al., 2016).

This result is however not in line with the data by Warchol-Biedermann *et al.* who surveyed 185 married males on four occasions (before diagnostic disclosure, two to three months after diagnostic disclosure, before the third appointment, and before the fourth appointment) (Warchol-Biedermann, 2021). In this study, it was reported that unintentionally childless males from couples with unexplained infertility undergoing fertility workup and treatment for the first time had significantly higher FertiQoL scores for the emotional, mind-body and relational domains compared to males from a couple suffering from male factor infertility, female factor infertility or mixed factor infertility before diagnostic disclosure and in the follow-up 2 to 3 months after the diagnostic disclosure. The score for the social domain of the FertiQoL was similar over all pathology groups and study visits (Warchol-Biedermann, 2021).

In another cohort study (n = 110), the degree of subjective wellbeing was measured using the von Zerssen symptom checklist resulting in an impairment score; healthy test persons fall close to 14.3. Impairment scores were compared between known infertility (female, male and couple) and unexplained infertility (n=5). No significant difference was found on the level of impairment between the different diagnostic groups of men and women respectively. Women attain the greatest rating of impairment (mean = 17.6) in the symptom checklist when, from a somatic point of view, they solely are responsible for the involuntary childlessness, followed by infertile women with an infertile partner and women with idiopathic infertility; women score the lowest rating if the cause of infertility is only attributed to their partner. Men from a couple with idiopathic sterility score the lowest compared to men in couples with known infertility (Kowalcek et al., 2001).

Recommendation

Healthcare professionals should be aware that - there is probably no difference in QoL between women with unexplained infertility versus women in couples with known causes of infertility, except when the cause of infertility is PCOS, where the QoL	Conditional ⊕OOO

is lower.

- QoL is probably higher in men from a couple with unexplained infertility compared to men from a couple with known causes of infertility except when the cause of infertility is men with a partner with PCOS, then the men from a couple with unexplained infertility have a lower QoL.

Justification

Current evidence indicates that there is probably no difference in QoL between women with unexplained and explained infertility, except for PCOS. For males with UI, QoL is probably higher compared to explained infertility. It is possible that QoL is impaired in the partner who is perceived to be responsible for infertility (Santoro et al., 2016).

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Annexes

Annex 1: Guideline development group

This guideline was composed of.

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DECLARATIONS OF INTEREST

All members of the guideline development group were asked to declare possible conflicts of interest by means of the disclosure forms (see *ESHRE Manual for Guideline Development*).

	Conflicts of interest
Daniela Romualdi	Honoraria from IBSA and Novo Nordisk
Baris Ata	Speakers fees from Merck, Gedeon Richter, Organon and Intas Pharma. Part of the advisory board of Organon Turkey and president of the Turkish Society of Reproductive Medicine.
Siladitya Bhattacharya	Editor and contributing author, Reproductive Medicine for the MRCOG, Cambridge University Press Speakers fees from Merck, Organon, Ferring, the Ostetric and Gynaecological Society of Singapore and the Taiwanese Society for Reproductive Medicine Part of the METAFOR and CAPE trials data monitoring committee
Ernesto Bosch	Research grants from Roche diagnostics, Gedeon Richter and IBSA Consulting fees from Merck, Ferring, MSD, Roche Diagnostics, IBSA, Abbott, Gedeon Richter Speaker's fees from Merck, Ferring, Gedeon Richter, IBSA, MSD, Roche Diagnostics Salary or position funding from IVI-RMA Valencia Ownership by stock or partnership from IVI-RMA Valencia and Mint diagnostics
Michael Costello	None declared.
Samuel Dos Santos-Ribeiro	Research grants from Roche Diagnostics, Organon, Theramex; Consulting fees from Ferring Pharmaceuticals, MSD and Organon; Speaker's fees from Ferring Pharmaceuticals, MSD/Organon, Besins, Theramex, Gedeon Richter; Stock or stock options from IVI Lisboa, Clínica de Reprodução assistida Lda; Equipment/medical writing/gifts from Roche Diagnostics and Ferring Pharmaceuticals.
Ksenija Gersak	None declared.
Roy Homburg	None declared.
Mina Mincheva	Consulting fees from Mojo Fertility Ltd
Robert Norman	Research grant from Australian National Health and Medical Research Council (NHMRC), Consulting fees from Flinders Fertility Adelaide, VinMec Hospital Hanoi Vietnam Speaker's fees from Merck Australia, Cadilla Pharma India, Ferring Australia Chair clinical advisory committee Westmead Fertility and research institute MyDuc Hospital Vietnam.
Terhi Piltonen	is part of the Research Council of Finland Research grants from Roche Diagnostics, Novo Nordics and Sigrid

	Juselius foundation Speaker’s fees from Gedeon Richter, Roche, Exeltis, Organon, Ferring and Korento patient organisation is part of NFOG, AE-PCOS society and several Finnish associations
Sara Somers	None declared.
Sesh Sunkara	Speaker’s fees from Merck, Ferring, MSD, Pharmasure
Harold Verhoeve	Consulting fees from Ferring Pharmaceuticals
Donia Scicluna	None declared.
Nathalie Le Clef	None declared.

Annex 2: Abbreviations

AA	Arachidonic acid
AMH	Anti-Müllerian hormone
AFC	Antral follicle count
ART	Assisted reproduction technology
ASA	Anti-sperm antibodies
AUC	Area under the curve
BBT	Basal body temperature
CAT	Chlamydia antibody testing
CC	Clomiphene Citrate
CCCT	Chlomiphene citrate challenge test
CI	Confidence interval
CT	Chlamydia Trachomatis
DFI	DNA fragmentation index
DHA	Docosahexaenoic acid
DNA	Desoxyribonucleic acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immuno Sorbent Assay.
ET	Embryo transfer
FRAP	Ferric reducing antioxidant power
GDG	Guideline development group
GPP	Good practice point
HR	Hazard ratio
HSG	Hysterosalpingography
HyCoSy	Hysterosalpingo-contrast-sonography
HyFoSy	Hysterosalpingo-foam-sonography
ICSI	Intracytoplasmic sperm injection
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IF	Immunofluorescence
IQR	Interquartile range
IU	International unit/infectious units
IUI	Intra-uterine insemination
IVF	In vitro fertilization
LH	Luteinizing hormone
LR	Likelihood ratio
MAR	Medically assisted reproduction
MAR test	Mixed antiglobulin reaction test
MD	Mean difference
MDA	Malondialdehyde
MIF	Micro immunofluorescence
NO	Nitric oxide
NOS	Nitric oxide synthase
NPV	Negative predictive value
NT	Nitrotyrosine
OR	Odds ratio

ORT	Ovarian reserve test
OS	Ovarian stimulation
OSCM	Oil-soluble contrast media
OSI	Oxidative stress index
PC	Protein carbonyl group
PCR	Polymerase Chain Reaction
PID	Pelvic inflammatory disease
PPV	Positive predictive value
RCT	Randomized controlled trial
RD	Risk difference
ROC-AUC	Receiver operating characteristic – area under the curve
ROS	Reactive oxygen species
RR	Relative risk/risk ratio
SCSA	Sperm chromatin structure assay
SDF	Sperm DNA fragmentation
SET	Single embryo transfer
SH	Thiol group
SIS	Saline infusion sonography
SMD	Standardized mean difference
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TAS	Total antioxidant status
TBARS	2-thiobarbituric acid-reactive substances
TOS	Total oxidant status
TVUS	Transvaginal ultrasound
UI	Unexplained Infertility
US	Ultrasound
WMD	Weighted mean difference
WSCM	Water-soluble contrast media

Annex 3: Recommendations for research on Unexplained Infertility

From the literature and discussion of the available evidence, several topics were identified for which evidence is inconsistent, insufficient or non-existing. For the benefit of patients with unexplained infertility, the GDG recommends that future research, where possible in well-designed RCTs, should focus on these research gaps.

The **top-3** of research recommendations with the highest priority identified by the GDG are:

1. Can a predictive model be developed, tested and validated to compare the outcomes of different management strategies for couples with UI?
2. What is the optimal ART for UI?
3. What is the value of performing current methods to assess sperm DNA integrity to predict clinical outcomes (pregnancy rates, live birth rates and miscarriage rates) in couples with UI?

Furthermore, the GDG would like to draw attention to the importance of research of male infertility. With a steady decline in sperm quality reported in the last 50 years, particularly sperm counts, male infertility has become a global public health issue (Levine et al., 2023, Levine et al., 2017). Over the past decade, there has been increasing evidence showing an association between male reproductive health and general health, with male infertility being proposed as a possible biomarker for current and future health (Burke et al., 2022, Chen et al., 2022, Del Giudice et al., 2021, Ventimiglia et al., 2015). However, up to 70% of male idiopathic and unexplained infertility cases remain with no aetiological factor (Punab et al., 2017, Salonia et al., 2023, Tüttelmann et al., 2018). MAR is routinely used for clinical management of male infertility when no causative factor is identified. Inadequate assessment of the causes of male infertility and the lack of strong evidence- supported treatment options puts a disproportionate burden on the female partner. Considering that the use of MAR is steadily increasing worldwide (Wyns et al., 2022), a paradigm shift in treatment of male factor infertility becomes essential (Björndahl, 2022, De Jonge and Barratt, 2019, Duffy et al., 2021). Therefore, re-focusing research efforts on addressing gaps in the understanding of male infertility, such as identifying new aetiological causes, clinical diagnostics, and MAR treatment options, will enable the development of more personalised therapeutic options to manage couple's infertility and improve reproductive outcomes.

Other research gaps that were identified are:

- What is the role of vaginal microbiota in UI?
- Can a predictive model for fertility based upon ovarian reserve tests be developed, tested and validated?
- In women at risk of age-related infertility, does standardized fertility assessment before attempting expectant management improve live birth rates?
- What causes UI?
- What is the relationship between luteal progesterone levels and spontaneous pregnancy?
- What is the impact of sperm DNA damage (evaluated via sperm DNA fragmentation tests and sperm chromatin condensation test) on the clinical management of couples with UI?
- What is the value of new and existing sperm function tests for prognosis or allocation to treatment?
- What is the role of lifestyle intervention?
- In women with otherwise unexplained infertility, does hysteroscopic removal of an endometrial polyp increase live birth rates?
- Can age-related infertility be prevented?

- What is the role of different endometrial biomarkers?
- What is the role of oxidative stress markers in semen in couples with UI?
- In women with a uterine septum and otherwise unexplained infertility does hysteroscopic resection increase live birth rates?
- What is the role of oxidative stress biomarkers in endometrial implantation?
- What is the relationship between regular menstrual cycles and proof of ovulation?
- In women with mild intrauterine adhesions and otherwise unexplained infertility does removal increase live birth rates?

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Annex 4: Methodology

Guideline development

The European Society of Human Reproduction and Embryology (ESHRE) guidelines are developed based on the Manual for ESHRE guideline development (N. Vermeulen, N. Le Clef, S. Mcheik, A. D'Angelo , K. Tilleman, Z. Veleva, W.L.D.M. Nelen, Manual for ESHRE guideline development, version 2019), which can be consulted on the ESHRE website (www.eshre.eu/guidelines). The principal aim of this manual is to provide stepwise advice on ESHRE guideline development for members of ESHRE guideline development groups. The manual describes a 12-step procedure for writing clinical management guidelines by the guideline development group, supported by the ESHRE methodological expert:

1. TOPIC SELECTION
2. GDG FORMATION
3. SCOPING
4. KEY QUESTIONS
5. EVIDENCE SEARCH
6. EVIDENCE SYNTHESIS
7. RECOMMENDATIONS
8. DRAFT FOR REVIEW
9. STAKEHOLDER REVIEW
10. EXCO APPROVAL
11. PUBLICATION
12. UPDATING / REVISING

The current guideline was developed and funded by ESHRE, which covered expenses associated with the guideline meetings (travel, hotel and catering expenses) associated with the literature searches (library costs, costs associated with the retrieval of papers) and with the implementation of the guideline (printing, publication costs). The National Health and Medical Research Council of Australia covered the expenses of the 2 Australian members of the panel (MC and RJN). Except for reimbursement of their travel expenses, GDG members did not receive any payment for their participation in the guideline development process.

The scope of the guideline and first version of the key questions were drafted by members of the ESHRE Special Interest Group (SIG) Reproductive Endocrinology, SIG Andrology, SIG Safety and Quality in ART and SIG Nursing and Midwifery and two representatives of the Monash University NHMRC Centre for Research Excellence in Women's Reproductive Health. We strived towards a balance in gender and location within Europe. Several online meeting of the guideline development group were organized to discuss the key questions and redefine them through the PICO process (patients – interventions – comparison – outcome). This resulted in a final list of 21 key questions. Based on the defined key words, literature searches were performed by the methodological expert (Dr. N. Le Clef). Key words were sorted to importance and used for searches in PUBMED/MEDLINE and the Cochrane library. We searched the databases from inception up to 24 October 2022.

Literature searches were performed as an iterative process. In a first step, systematic reviews and meta-analyses were collected. If no results were found, the search was extended to randomized controlled trials, and further to cohort studies and case reports, following the hierarchy of the levels of evidence. References were selected or excluded by the methodological expert and expert GDG member based on title and abstract and knowledge of the existing literature. If necessary, additional searches were performed in order to get the final list of papers. It is not within ESHRE's remit to conduct a formal investigation or to draw formal conclusions regarding the misconduct of an individual or group of individuals or to determine whether a published article should be retracted. However, papers that are withdrawn, have a published editorial note of concern or a published expression of concern have been excluded from the guideline. In future revision or update of the guideline, the GDG will actively verify the status of all the referenced studies.

The quality of the selected papers was assessed by means of the quality assessment checklist, defined in the ESHRE guideline manual. Furthermore, the evidence was collected and summarized in an evidence table according to GIN format (http://www.g-i-n.net/activities/etwg).

The quality assessment and evidence tables were constructed by the expert GDG members. Summary of findings (SoF) tables were prepared following the GRADE approach for intervention studies which reported the critical outcomes. The critical outcomes for this guideline were: live full-term singleton birth, live birth, ongoing pregnancy rate, multiple pregnancies/multiple births.

GDG meetings were organized to discuss the draft recommendations and the supporting evidence and to reach consensus on the final formulation of the recommendations. In a final step, all evidence and recommendations were combined in the evidence-based guideline: "Unexplained Infertility".

LIST OF KEY QUESTIONS

DEFINITION OF UNEXPLAINED INFERTILITY

Question I.1 After how many months of unprotected intercourse should a couple be defined as infertile?

Question I.2 Should frequency of sexual intercourse affect the definition of UI?

Question I.3 Should female or male partner's age affect the definition of UI?

Question I.4 Should couples with mild infertility factors be included in the definition of UI?

DIAGNOSIS OF UNEXPLAINED INFERTILITY

Question II.1 Which is the reliability and convenience of methods to confirm regular ovulation?

Question II.2 What is the reliability of parameters detecting good oocyte/corpus luteum quality?

Question II.3 Should one or more tests of ovarian reserve be included in the diagnostic work-up?

Question II.4 What is the accuracy of commonly used tests of tubal patency?

Question II.5a Which diagnostic procedures should be performed to confirm a normal uterine structure/anatomy, uterine wall/myometrium?

Question II.5b Which additional diagnostic procedures should be performed to confirm an anatomically normal uterine cavity?

Question II.6 Should women undergo a laparoscopy before being diagnosed with UI?

Question II.7 What is the need for female lower genital tract investigations?

Question II.8 Should men undergo additional diagnostic procedures to confirm normal genito-urinary anatomy before being diagnosed with UI?

Question II.9 Is there added value of additional tests in the male with normal WHO semen analysis?

Question II.10 Should there be additional evaluations of possible systemic cause of UI in the couple?

Treatment of unexplained infertility

Question III.1 What is the value of expectant management compared to active treatment for patients with UI?

Question III.2a If active treatment is pursued, which type of active treatment for UI?

Question III.2b What is the value of IVF versus ICSI?

Question III.3 What is the value of mechanical-surgical procedures?

Question III.4 What is the effectiveness of alternative therapeutic approaches?

Quality of life

Question IV.1 Is there a difference in QoL for patients with unexplained versus explained infertility?

Formulation of recommendations

We labelled the recommendations as either ''strong'' or ''conditional'' according to the GRADE approach. Suggested interpretation of strong and conditional recommendations by patients, clinicians and health care policy makers is as follows:

Implications for	Strong recommendation	Conditional recommendation
Patients	Most individuals in this situation would want the recommended course of action, and only a small proportion would not	The majority of individuals in this situation would want the suggested course of action, but many would not
Clinicians	Most individuals should receive the intervention Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences	Recognise that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences Decision aids may be useful in helping individuals to make decisions consistent with their values and preferences
Policy makers	The recommendation can be adopted as policy in most situations	Policy making will require substantial debate and involvement of various stakeholders

For each recommendation it is mentioned whether it is strong or conditional and what the quality of the supporting evidence was. In the justification section, more data are provided on the considerations taken into account when formulating the recommendations: balance between desirable and undesirable effects, certainty of the evidence of effects, certainty in how people value the outcome, acceptability and feasibility of the intervention. Impact on health equity and resource impact were only discussed where relevant. For interventions where there was no evidence from studies focussing on unexplained infertility specifically to support the recommendation, the quality of the evidence was automatically graded as very low (+OOO).

Strategy for review of the Guideline draft

After finalization of the guideline draft, the review process was initiated. The draft guideline was published on the ESHRE website, accompanied by the reviewers' comments form and a short explanation of the review process. The guideline was open for review between 12 December 2022 and 30 January 2023.

To notify interested clinicians, we sent out an invitation to review the guideline by email to all ESHRE members.

Selected reviewers were invited personally by email. These reviewers included:

- *Coordinators and deputies of the ESHRE SIG Reproductive Endocrinology, SIG Andrology, SIG Reproductive Surgery, SIG Safety and Quality in ART and SIG Nursing and Midwifery.*
- *Contact persons of patient organizations across Europe.*
- *Contact persons of international and national societies focused on IVF/ICSI across Europe.*

All reviewers are listed in annex 5. The Reviewer comments processing report, including further information on the review and a list of all comments per reviewer with the response formulated by the GDG will be published on the ESHRE website.

Guideline Implementation strategy

The standard dissemination procedure for all ESHRE guidelines comprises publishing and announcement.

Each guideline is published on the ESHRE website and in Human Reproduction Open. The announcement procedure includes a news item in "Focus on Reproduction", a newsflash on the ESHRE website homepage and a short presentation at the ESHRE Annual meeting. All participants in the annual ESHRE meeting will be informed about the development and release of new guidelines; all related national societies and patient organizations are informed about the guideline release. They are asked to encourage local implementation by, for instance, translations or condensed versions, but they are also offered a website link to the original document.

Patient versions of the guideline will be developed by a subgroup of the GDG together with patient representatives. The patient version is a translation of the recommendations in everyday language, with emphasis on questions important to patients. It aims to help patients understand the guideline's recommendations and facilitates clinical decision-making.

To further enhance implementation of the guideline, the members of the GDG, as experts in the field, will be asked to select recommendations for which they believe implementation will be difficult and make suggestions for tailor-made implementation interventions (e.g. option grids, flow-charts, additional recommendations, addition of graphic/visual material to the guideline).

Further translation tools will be developed by CRE in partnership with ESHRE.

SCHEDULE FOR UPDATING THE GUIDELINE

The current guideline will be considered for revision in 2027 (four years after publication). An intermediate search for new evidence will be performed two years after publication, which will inform the GDG of the necessity of an update.

Every care is taken to ensure that this publication is correct in every detail at the time of publication. However, in the event of errors or omissions, corrections will be published in the web version of this document, which is the definitive version at all times. This version can be found at www.eshre.eu/guidelines.

For more details on the methodology of ESHRE guidelines, visit www.eshre.eu/guidelines

Annex 5: Stakeholder consultation

As per routine development procedures, the guideline draft was open for review for 6 weeks, between 12 December 2022 and 30 January 2023. All reviewers, their comments and the reply of the guideline development group are summarized in the review report, which is published on the ESHRE website as supporting documentation to the guideline. The list of representatives of professional organization, and of individual experts that provided comments to the guideline are summarized below.

Organisation	Country	Representative
German Society of Andrology (DGA e.V.) German Society of Urology, working group Andrology	Germany	Sabine Kliesch
Institute of Reproductive Medicine, Kolkata	India	Pratip Chakraborty
Centre for Human Reproductive Science, Birmingham Health Partners, The University of Birmingham	UK	Jackson Kirkman-Brown Meurig Gallagher
Reproductive medicine AmsterdamUMC, The Netherlands and the Netherlands Cochrane Gynaecology&Fertility	The Netherlands	J.A. Wessel, Elena Kostova, Monique Mochtar, Madelon van Wely, Femke Mol, Mariette Goddijn
European Academy of Andrology	Europe	Dimitrios G. Goulis Giovanni Corona
German Society for Reproductive Medicine (DGRM) and URZ	Germany	Ziller V., Goeckenjahn M., Köhn F.-M.., Hancke K. , Sonntag B.
Bundesverband Reproduktionsmedizinischer Zentren (BRZ)	Germany	Ulrich A. Knuth, Michael Ludwig
German Society of Human Reproductive Biology	Germany	Verena Nordhoff
Unexplained Infertility Guideline Australian Adaptation Committee	Australia	

Reviewer	Country
Joel Bernstein	Australia
Gustavo Botti	Argentina
Maruf Siddiqui	Bangladesh
Jean Calleja-Agius	Malta
Hunida Elmegrab	Libya
Bulent Tandogan	Turkey
Carlos Calhaz-Jorge	Portugal
Liliana Ramos	The Netherlands

Michael Morris	Switzerland
Adam Balen	UK
George Lainas	Greece
Mahmoud A Abdel-Aleem	Egypt
Marco Sbracia	Italy
Mario Sousa	Portugal
Maria Elisabetta Coccia	Italy
Michael Grynberg	France
Exalto N. Emanuel MH.	The Netherlands
Ben Mol	Australia
Mitranovici Melinda Ildiko	Romania
Panayotidis Costas	Greece
Nusrat Mahmud	Bangladesh
Aboubakr Mohamed Elnashar	Egypt
Mira Töyli	Finland
Petya Andreeva	Bulgaria
Kalmantis Konstantinos	Greece
Åsa Magnusson	Sweden
Christina Bergh, Jan Bosteels Frank Broekmans Astrid Cantineau Arri Coomarasamy Vinh Dang Annemieke Hoek Joop Laven Rong Li Abha Maheswari Ben W. Mol Anja Pinborg Annika Strandell Chris Venetis Lan Vuong Madelon van Wely	Sweden Belgium The Netherlands UK Vietnam China Australia Denmark
Maria Schubert	Germany
Monica Varma	India
Christophe Blockeel	Belgium
Priya Bhide	UK
Lars Björndahl	Sweden