Evidence-based Investigations for Subfertility

1 POINT

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INTRODUCTION

According to the World Health Organization (WHO), subfertility is 'a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse'.[1] The prevalence of subfertility varies widely depending on the definition used,[2] but undoubtedly it is a global problem of which nearly 72.4 million couples are estimated to have fertility problems.[3] The consequences of subfertility are manifold and can include psychological and social impact on the individuals as well as the cost burden on the health care system.

Evaluation of the subfertile couple should therefore be conducted in a systematic, expeditious, and cost-effective manner to identify all relevant factors, with initial emphasis on the least invasive methods for detection of the most common causes of infertility.[4] Approximately 84% percent of couples conceive within 1 year of trying, and about 92% do so within 2 years.[5] It is recommended that subfertility evaluation should be initiated after 1 year of attempted conception in women under age 35, and earlier evaluation is warranted in women over age 35 years or women with oligo-/amenorrhoea or known risk factors for infertility, such as endometriosis, a history of pelvic inflammatory disease, or reproductive tract malformations.

Subfertility is a worldwide problem – 72.4 million couples are estimated to have fertility problems globally.

There are multiple causes of subfertility and most are related to ovulatory disorders, tubal damage, uterine or peritoneal disorders, endometriosis, and factors in the male causing infertility. In about 25% of cases the cause is unexplained in view of normal semen analysis, ovulation and patent tubes, and in nearly 40% of infertile couples, both the man and the woman are affected. It is therefore of paramount importance that the couples are evaluated together and separately, with the aim to establish presence of subfertility, to determine the underlying cause and to provide appropriate counseling and treatment accordingly.[6]

INITIAL ASSESSMENT

Initial consultation with the subfertile couple should include a complete medical and reproductive history and physical examination, preconception counseling, and instruction on the optimization of in-

Table 1. Focused history and examination of the couple

	Female	Male
History	*Fertility history* • Previous pregnancies and outcomes • Duration of infertility • Previous investigations/treatment *Menstrual history* • Cycle regularity • Dysmenorrhoea *Medical history* • Systemic illness • Medications/on folic acid • Genetic disease *Gynaecological history* • Pelvic inflammatory disease • Sexually transmitted disease • Endometriosis/dyspareunia • Previous contraception • Cervical smears *Surgical history* • Complicated appendicitis • Ovarian cystectomy *Social history* • Smoking/alcohol • Recreational drugs	*Fertility history* • Previous pregnancies • Duration of infertility • Puberty onset *Medical history* • Systemic illness – diabetes • Genetic disease • History of mumps • Sexually transmitted disease • Medications *Surgical history* • Orchidopexy, cryptorchidism • Testicular torsion *Sexual history* • Erections/ejaculations • Frequency of intercourse *Social history* • Smoking/alcohol • Recreational drugs *Occupation*
Examination	Body mass index Thyroid and breast examination Secondary sexual characteristics Presence of hirsutism Pelvic examination and examination of vagina and cervix Determination of uterine size, shape and mobility Check for adnexal masses, tenderness or nodularity	Body mass index Secondary sexual characteristics *If abnormal SA:* Palpation and measurement of testes Presence of vas Examination of epididymides Check for varicocoele

tercourse. A summary of the history and examination of an infertile couple is given in Table 1.

For all women seeking fertility, susceptibility to rubella should be checked. Maternal rubella infection in the first trimester of pregnancy can result in severe congenital fetal abnormalities[7] and it is easily preventable by pre-conception vaccination. Screening for populations at risk for specific inheritable diseases should be performed. Thalassaemia is one of the world's most common single-gene disorders, and prevalence is especially high in Asia, Middle East, and Mediterranean region. Women at risk should have their mean corpuscular volume (MCV) checked as a screening test. If a woman is found to be a genetic carrier, the male partner should also be offered testing. Up-to-

date cervical smear of the women should also be ensured.

INVESTIGATIONS

Evaluations of Male Subfertility

Semen Analysis

A male factor is solely responsible in approximately 20% of subfertile couples and contributes in another 30-40% of couples.[8] Semen analysis (SA) is the cornerstone of the evaluation following history taking and physical examination, and it helps to define the severity of the male factor. A minimum of at least one semen sample should be collected for evaluation after an abstinence interval of 2 to 5 days. A longer period of abstinence can lead to reduced motility in normospermic samples.[9] The semen sample provides information on semen volume as well as sperm concentration, motility, and morphology, and the analysis should be performed according to WHO criteria.[10] The reference values are listed in Table 2, and each laboratory should have a quality control program that conforms to the standards.

Table 2. World Health Organization semen analysis reference values

Parameter	Lower reference limit
Semen volume	1.5 mL
Sperm concentration	15 million
Total sperm number	39 million
Progress motility	32%
Total motility	40%
Sperm morphology	4%

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Assessment for the presence of autoimmune anti-sperm antibodies should no longer be the standard part of SA because the significance of its presence is unclear and there is no effective treatment in terms of improving male fertility.[5] Limitations of SA include individual fluctuations and a substantial overlap between fertile and subfertile values.[11] A repeat semen analysis should be performed, ideally at least 3 months after the initial sample, if the result of the first analysis is abnormal.[5] If azoospermia or severe oligospermia is reported in the initial SA, a repeat test should be undertaken within 2 to 4 weeks.

Routine use of specialized clinical tests on semen and sperm including sperm function tests, computer-assisted seminal analysis, and sperm DNA fragmentation tests in the clinical evaluation of male factor infertility is currently not recommended.[5,12] Although sperm DNA damage may associate with poor reproductive performance, current methods for assessing sperm DNA integrity do not reliably predict treatment outcomes. Sperm function tests also vary in their ability to detect defects in the complex processes leading to fertilization, and therefore are of limited use from a practical point of view. These tests should be reserved as research tools rather than as routine clinical tests.

Additional Testing

If hypogonadism is suspected based on the SA (severe oligospermia with sperm concentration below 10 million/mL or azoospermia), evaluation of serum follicle-stimulating hormone (FSH) and total testosterone concentrations is indicated.[13] If severe oligospermia (<5 million/mL) is found and the physical examination does not reveal signs of obstruction, the American Society for Reproductive Medicine (ASRM) suggested a genetic workup as the prevalence of chromosomal abnormalities is inversely proportional to sperm count.[14,15] However, in Hong Kong, the prevalence rates of chromosomal abnormalities and Y-microdeletions were only 1.5% in men with sperm concentration >2 and <5 million/mL compared to 13.9% in men with sperm concentration >0 and ≤2 million/mL; hence genetic workup is only recommended in infertile men with sperm concentrations of 2 million/mL or lower.[16]

A thorough evaluation by an urologist or reproductive endocrinologist is warranted for men with abnormal semen parameters, physical examination or endocrine/genetic profile.

Evaluation of female subfertility

Assessment of Ovulation

Pregnancy is the ultimate test of normal ovulation, but it only occurs in around 25% of cycles in women who have intercourse during the peri-ovulatory period.[17] Ovulatory disorders account for up to 25% of subfertility causes, and it is usually reflected in menstrual irregularities, typically cycles of long duration or complete absence of cycles. Common

Table 3. Common causes of ovulation disorders

Hypogonadotrophic hypogonadism
• Idiopathic
• Kallmann's syndrome
• Excessive weight loss, exercise, stress, drugs
• Pituitary tumour, pituitary necrosis or thrombosis
Normogonadotrophic normogonadic ovarian dysfunction
• Polycystic ovary syndrome
Hypergonadotrophic hypogonadism
• Genetic, Turner's syndrome
• Autoimmune
• Surgical menopause, post radiotherapy/chemotherapy
• Idiopathic
Other endocrinopathies
• Hyperprolactinaemia
• Thyroid dysfunction
• Androgen excess

causes of ovulatory disorders are listed in Table 3. Regular menstrual cycles in the range of 22 to 35 days are usually indicative of ovulation, as does the presence of premenstrual symptoms.[18] However, confirmation with mid-luteal (day 21 of a 28-day cycle) serum progesterone is recommended for women with regular menstrual cycles and more than 1 year's infertility.[5] Values range from 16-28 nmol/L as the lowest limit indicative of ovulation. For women with irregular cycles, this test may need to be done later in the cycle and repeated weekly until the next menstrual period.

Testing in women with amenorrhoea or oligomenorrhoea should also involve FSH, prolactin, and thyroid-stimulating hormone (TSH) levels, to help determine the underlying cause of anovulation. In asymptomatic women with regular cycles, these hormones should not be checked routinely. Pelvic ultrasound will be performed for polycystic ovary morphology.

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In ovulatory cycles, morning basal body temperature (BBT) often increases as the cycle progresses from the follicular phase to the luteal phase, giving the biphasic BBT recordings. However, not all women with ovulatory cycles will have clearly documented biphasic BBT.[19] Although BBT charting is a simple and inexpensive means of documenting ovulation, it does not reliably predict ovulation[20] and is therefore not recommended.[5] In recent years, women have largely replaced BBT charting by the less tedious, more easily available urinary luteinizing hormone (LH) kit to detect ovulation. During ovulatory cycles, an LH surge can be detected in the urine 14-48 hours before ovulation.[21] Urinary LH kits can accurately predict ovulation,[22] but reliability can be variable among different commercial kits[23] and one should be aware of potential false-positive and false-negative results when interpreting. Daily serum LH measurements to detect LH surge is considered impractical in clinical setting.

Monitoring the daily growth of the pre-ovulatory follicle by pelvic ultrasound and eventually the demonstration of collapse of the growing follicle and appearance of fluid in cul-de-sac indicates ovulation. Due to the labour intensive nature and high cost, this is not considered the preferred method of ovulation detection.

Assessment of Tubal Patency

Different screening strategies are available in assessing tubal patency and diagnosing tubal subfertility, and the ones that have been studied and used widely include laparoscopy with chromotubation, hysterosalpingography, hysterosalpingo-contrast sonography, and chlamydial serology. More sophisticated tests like transvaginal hydrolaparoscopy, salpingoscopy, falloposcopy and fertiloscopy require special expertise and their diagnostic accuracy and prognostic ability require further evaluation. Each diagnostic test has its own benefits and limitations and therefore the selection of a particular test should be individualized. Invasive tubal patency test should only be offered after taking into account the overall treatment needs of the women,

In recent years, urinary luteinizing hormone (LH) test kits have largely replaced more tedious, less accessible methods of assessment of female ovulation.

and testing can be avoided in those who require in vitro fertilization (IVF) treatment regardless.

Genital chlamydia trachomatis infection is recognized as the single most common cause of tubal peritoneal damage,[24] and therefore chlamydia serology has been used as a screening test for tubal damage in infertile women. The sensitivity and specificity of C trachomatis antibody test (CAT) varies depending on the assays used and the cut-off value used to define a positive result.[25] It should not be regarded as a diagnostic test but as a screening test to facilitate decisions on which women should proceed with further investigations.

Hysterosalpingography (HSG) refers to the radiographic evaluation of the uterine cavity and fallopian tubes after injection of a radio-opaque medium through the cervix. It is widely available and provides information about tubal patency, site of occlusion, outline of the lumen, as well as the contour of the uterine cavity. In addition, a therapeutic benefit has been observed with the use of oil-soluble contrast medium when compared to no intervention and water-soluble contrast medium,[26] possibly related to the flushing of debris from the tubal lumen. HSG has sensitivity and specificity of 53% and 87% respectively,[27] and the moderate sensitivity is likely related to tubal spasm during dye injection and intra-observer variability. It should be offered as first line tubal investigation for subfertile women with no risk factors,[5] after explaining to them the risk of pelvic infection, anaphylaxis and radiation exposure.

Hysterosalpingo-contrast sonography (HyCoSy) is an alternative imaging technique to the HSG. It involves transvaginal ultrasound and injection of sonographic contrast medium into the uterine cavity. It has the advantage over HSG of simultaneous examination of the ovaries and myometrium, and avoidance of radiation exposure. A recent systematic review and meta-analysis showed that HyCoSy had similar sensitivity and specificity as HSG when compared to diagnostic laparoscopy.[28] Where appropriate expertise is available, HyCoSy can be considered as an effective alternative to HSG.[5]

Diagnostic laparoscopy with chromotubation (injection of a blue dye

A detailed and focused history examination of a couple affected by fertility problems will aid initial investigations and treatment.

through the cervix and visualization of spillage out of the fimbrial end of the tubes) is considered to be the gold-standard test for tubal patency. It offers the additional benefits of assessing the whole pelvis and opportunistic treatment of possible pathologies like minimal/mild endometriosis and peri-tubal adhesions will provide some therapeutic benefits.[29] However, given its invasiveness and requirement for general anaesthesia, it should not be routinely offered but only to those with inconclusive/abnormal HSG result and/or risk factors which include history of complicated appendicitis, pelvic surgery, ectopic pregnancy, pelvic inflammatory disease, and endometriosis.[5,30]

Uterine Factor Evaluation

Uterine cavity abnormalities such as adhesions, endometrial polyps, submucosal fibroids and uterine septum have been found in 10-15% of women seeking treatment for fertility problems.[31] However, the causal relationship between these uterine abnormalities and subfertility has not been properly established. Ultrasonography for uterine evaluation is therefore not a mandatory investigation, although many clinicians perform scans routinely nowadays due to its easy access and wide availability. Any suspicious findings on ultrasonography will require further evaluation with saline infusion sonohysterography or hysteroscopy, but these invasive tests should not be considered as a routine investigation. Currently, insufficient evidence exists to support routine surgical treatment of uterine abnormalities in improving pregnancy rates.[32]

In the past, endometrial biopsy had been a routine part of the fertility evaluation to check for ovulation and luteal phase defect by "dating" the endometrium using traditional histologic criteria.[33] However subsequent studies have not proved this test to be a useful discriminator between fertile and infertile populations,[34] and it is no longer a recommended part of the infertility evaluation.[4,5]

Cervical Factor Evaluation

The presence of motile sperm in perio-vulatory cervical mucus on postcoital

test (PCT) was the traditional method for diagnosis of cervical factor subfertility. However this test has poor diagnostic potential and predictive value.[35] Moreover, a randomized controlled trial comparing the outcome of subfertility investigations with and without PCT showed no difference in cumulative pregnancy rate at 24 months.[36] These limitations have render PCT unnecessary for subfertility evaluation.[4,5]

Ovarian Reserve Evaluation

Ovarian reserve, the size of the oocyte pool, reflects the reproductive potential of a woman and it is closely associated with female age, which is the single most important factor influencing reproductive outcome. Tests utilized to assess ovarian reserve include cycle day 3 FSH, an early follicular phase antral follicle count via transvaginal ultrasonography, or a serum anti-mullerian hormone (AMH) level.[4] Unfortunately none of these tests is reliable for predicting pregnancy potential in IVF treatment or in spontaneous pregnancy.[37,38] Ovarian reserve tests do not establish a diagnosis of subfertility, and tests should only be done prior to assisted reproductive technology (ART) treatment to predict the likely ovarian response to stimulation with exogenous gonadotropins.

CONCLUSION

Subfertility is a common condition afflicting couples, and both partners should be evaluated promptly once the diagnosis of subfertility has been established. A detailed and focused history and examination of the couple will help facilitate initial investigations and treatment. The basic subfertility evaluation consists of semen analysis, assessment of ovulatory status, and determination of tubal patency. Additional testing beyond these basics should be pursued only when clinically indicated or for research purposes.

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