CHAPTER 17

# Laboratory Procedures for Evaluating a Patient for Assisted Conception

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## INTRODUCTION

Infertility is defined as failure to conceive after 12 months of unprotected intercourse. Infertility affects one in six to seven couples. After one year of unprotected intercourse, 85% to 90% of the couples will succeed to conceive. Among the remaining couples, 5% will conceive during the second year [1].
New reproductive technologies are unavailable, scarcely available or very costly in so far the large majority of the population cannot afford infertility treatment at all in developing and low resource settings[2] despite the fact that developing countries have large reservoir of infertility problems of which bilateral tubal occlusion is the most important one[3], a condition that is potentially treatable by assisted reproductive technologies (ART). However, policy makers in developing countries are confronted by major health problems such as high maternal mortality, malnutrition and infectious diseases and hence find it difficult to give ART the attention it deserves and thus specialist of ART in developing countries are confronted with balancing standards with cost effectiveness of the numerous procedures of ART.

Laboratory evaluation of patients for ART constitutes a major component of ART because it is involved in diagnosis, monitoring, treatment and also stratifying the type ART to be offered to the patient either intrauterine insemination, in vitro fertilization or intracytoplasmic sperm injection. Laboratory investigations bear a significant burden in terms of cost of ART and thus an important area in low resource countries to look inwards for cheaper protocols.

This chapter is aimed at discussing the various laboratory evaluation modalitiesWhy is laboratory investigations important in IVF workup? in ART, highlighting challenges and alternative modalities in low resource countries.

### LABORATORY EVALUATION OF PATIENTS FOR ART

When couples present at ART centres for treatment, they often would have been evaluated previously to arrive at a diagnosis indicating ART however some

patients present directly to the ART centres for evaluation. Preliminary investigations of infertile couples that may establish a diagnosis necessitating ART are treated in other aspects of this text. This chapter would therefore focus on the specific investigations relevant for ART workup, stratifying the type of ART needed, treatment and monitoring. The laboratory evaluation will be categorized into male and female laboratory evaluation.

## MALE LABORATORY EVALUATION

The male laboratory investigations are faster, more convenient, non invasive and cheaper than those of the female thus where possible it is more cost effective to start with the male partner even though men in our environment are reluctant to subject themselves to investigations[4].

The basic male investigation begins with a detailed history and physical examination. Semen analysis and serum hormonal profile represents the first line laboratory investigations. The aim is to identify the underlying causes of male factor that may be correctable to enhance the fertility status.

The male laboratory evaluation will be discussed as general, infection screening, semen, hormonal assay and others as in Table 1.

**1: male laboratory evaluation**

| | |
|---|---|
| General | Haemoglobin.<br>Blood group & Rhesus status.<br>Haemoglobin genotype<br>Blood sugar (R) if abnormal-Fasting & 2hr Post Prandial blood sugar.<br>Over weight-check fasting lipid profile. |
| Infection screening | HIV antibodies.<br>Hepatitis B<br>Hepatitis C<br>VDRL |
| Semen | Analysis.<br>Culture.<br>24 hour survival<br>DNA fragmentation tests |
| Azoospermia/ severe oligo, teratospermia | LH,FSH, Testosterone<br>Prolactin<br>TSH<br>Scrotal ultrasound to assess volume and exclude hydrocele, varicocele |
| | |
| Others | Genetic testing<br>Karyotype<br>Microdeletion of Y chromosones |

## GENERAL

The general laboratory investigation in male partner includes hemoglobin level estimation and should be at least 10g. Blood grouping and genotype is important in case the female partner is Rhesus negative and HB AS, AC & SS respectively so that genetic manipulations and karyotyping can be done to have a normal offspring even though the technology is not readily available in developing countries where we have high reservoir of the problems. Blood sugar testing is very important in males because Diabetes can cause erectile dysfunction and retrograde ejaculation causing difficulty in semen collection for analysis and ART. Assessing the lipid profile of the male partner especially in overweight or obese patients is recommended to prevent its attendant sequelae.

## INFECTION SCREENING

Males should be routinely screened for Human immunodeficiency virus (HIV) and should be offered the options of counseling regarding the implication of this blood test. Similarly, it is essential to test for hepatitis B (HBV) and hepatitis C virus (HCV) antibodies. While a few years ago it was common practice to deny ART treatment to patients who tested positive to HIV, the current trend due to low vertical transmission of the disease if appropriate measures are taken is that IVF or ICSI is a reasonable option if required.

## SEMEN

The first line laboratory investigation for male infertility includes semen analysis. There are inter-laboratory and intra-individual variations in semen analysis. Thus abnormal semen analysis result should be repeated at least one month later to confirm the diagnosis[5].

The results of the semen analysis conducted as part of an initial assessment should be compared with the WHO 2010[6] reference values as described in relevant section on male infertility.

If the semen analysis is abnormal, further tests are requested to discriminate pituitary-hypothalamic axis from testicular dysfunction and

genital obstruction. Serum FSH and total testosterone measurements should be performed in all cases of oligospermia. Additional hormonal evaluation such as LH, prolactin and TSH should be requested if the clinical findings suggest a specific pathology. Low levels of FSH, LH (< 2IU/l), and testosterone in the context of low sperm concentration suggest hypogonadotropic hypogonadism. Though this later entity is not a common cause of male infertility. In case of low sperm concentration due to primary testicular failure, the testosterone will be low while FSH and LH will be high (> 8IU/l)

## GENETIC TESTING

In case of testicular failure, genetic testing including karyotype analysis and Y - chromosome micro-deletion should be performed. A karyotype analysis can diagnose numeric chromosomal abnormalities e.g klinefelter syndrome. Men with severe oligospermia or non-obstructive azoospermia should have karyotype before IVF with ICSI using their sperm. The most common genetic abnormalities that cause decrease sperm production are numeric and structural chromosome aberrations and Y chromosome micro-deletions. Men with obstructive azoospermia due to congenital bilateral absence of the vas deference (CBAVD) most commonly have an abnormality of the cystic fibrosis transmembrane conductance regulatory gene (CFTR). Micro-deletion of sections of Y chromosome can be found in 10 -15% of men with azoospermia or severe oligospermia. This occurs in region of the long arm of the Y chromosome (Yp11) known as the azoospermic factor (AZF) regions, which contain necessary genes for spermatogenesis. AZFa is the proximal region, AZFb is the central region and AZFc is the distal region of the arm. The DAZ (deleted in azoospermia) region is in the AZFc region. The location of Y chromosome micro-deletion may significantly affect spermatogenesis. Men with AZFc deletion may have severe oligospermia or azoospermia with enough testicular sperm for retrieval. Men with AZFa or AZFb deletions have azoospermia and a poor prognosis for testicular sperm retrieval. Sons of men with Y micro-deletion will inherit the abnormality and may be infertile. Y chromosome analysis should be offered to men with non-obstructive azoospermia and severe oligospermia before IFV with ICSI using their sperm.

In low resource settings like ours we face tremendous challenges as regards the evaluation of male infertility. Many of the special tests and genetic screening discussed above are not available in developing countries posing serious challenge in the management of severely abnormal semen parameters more often samples are taking outside the country for such evaluations which is very costly obviating the need to have it done in some instances where patient opt out in the process of counseling them to have the tests done.

## FEMALE LABORATORY EVALUATION

Laboratory evaluation of the female is preceded by a detail history and complete physical and systemic examination to be able to elucidate the crux of the problem. A complete evaluation of the female reproductive tract involves cervical, uterine, endometrial, tubal, peritoneal and ovarian factors. Since thyroid disease and hyperprolactinaemia can cause menstrual abnormalities and infertility serum thyroid stimulating hormone (TSH) and prolactin levels should be checked first before instituting further investigation. Female laboratory evaluation would be discussed systematically as in table 2 as General, infection screening, uterine, ovarian, peritoneal, endocrine and others.

**Table 2: female laboratory evaluation**

| | |
|---|---|
| General | Haemoglobin.<br>Blood group & Rhesus status.<br>Haemoglobin genotype<br>Blood sugar (R) if abnormal Fasting & 2hr<br>Post Prandial blood sugar, oral glucose<br>tolerance test (OGTT).<br>Over weight check fasting lipid profile. |
| Infection screening | HIV antibodies.<br>Hepatitis B<br>Hepatitis C<br>VDRL<br>Rubella status |
| Uterine | Hysteroscopy |
| Ovarian | FSH, progesterone, estrogen, LH,<br>anti_Mullerian hormone (AMH)<br>Inhibin B<br>ultrasound |
| Other Endocrine tests | Prolactin, TSH, Testosterone |
| Tubal | HSG |
| Peritoneal/Tubal | Laparoscopy |
| Others | Karyotype<br>MRI |

## GENERAL

As in the male evaluation but in addition OGTT and lipid profile is important in obese and polycystic ovarian syndrome (PCOS) patients.

## INFECTION SCREENING

As in the male evaluation but in addition Rubella status is very important. The presence of Rubella antibody indicate that the patient is immune for rubella however if negative for that antibody then the patient has to be immunized for rubella which is not available in most developing countries.

## UTERINE

Intrauterine lesions including endometrial polyp, submucous fibroid, adhesions or uterine septum can interfere with implantation and compromise pregnancy rates in ART. This can be evaluated using hysteroscopy which is a method for direct visualization of the endometrial cavity, which is commonly performed as an office procedure using local anesthesia i.e. para-cervical block. Hysteroscopy is a definitive method for both the diagnosis and treatment of intrauterine pathology, which is likely to have an effect on implantation after embryo transfer. Hysteroscopic surgery can also be used for treatment of intrauterine pathology such as uterine synechiae, endometrial polyps, submucous myomas and lysis of intrauterine adhesions. During hysteroscopy an opportunity is used to do a dummy embryo transfer (ET). However, the hysteroscopic facility may not be readily available in all centres and where available may be just for diagnostic purpose. In developing countries where hysteroscopy may not be readily available a transvaginal ultrasound is an inexpensive, easy, well tolerated procedure. Its sensitivity to detect intrauterine lesion ranges from 56% to 80% [8]. However, it may be difficult to detect the presence of sub-mucosal fibroids in the presence of multiple fibroids, endometrial polyp in the presence of thick endometrium, and to diagnose synechiae or uterine malformations.

Besides evaluating tubal patency for patients for IUI, HSG can also provide assessment of the uterine cavity. However, the findings of intrauterine filling defects could also be due to air bubbles, mucus, and menstrual debris. False negative findings can result from excessive amount of contrast media obliteration shadows caused by small lesions. Compared to hysteroscopy, HSG has a sensitivity of 60% to 98% and specificity from 15% to 80% with high rates of false positive and false negative [9]. HSG provides an idea of uterine cavity but it should not be used specifically to evaluate uterine cavity.

## OVARIAN

A major factor in successful IVF treatment is the ability of the ovary to respond to gonadotropin stimulation and to develop several follicles. That response reflects the ovarian function or ovarian reserve. Serum measurements of FSH and estradiol (E2) are performed in the early follicular phase from day 2 to 5 of the cycle. FSH is an indirect marker of ovarian reserve. It predicts ovarian response to FSH stimulation. FSH is down regulated by E2. Accordingly, low FSH value could be encountered when the level of E2 is high. Both hormones should be evaluated together. The upper threshold of FSH varies between 10 and 25IU/l [10]. The accuracy of FSH to predict poor response is more accurate at very high threshold levels.

Another marker for ovarian reserve is serum anti- mullerian hormone (AMH). It is a glycoprotein produced only by the ovaries in women. AMH expression is absent in primordial follicles and appears in granulosa cells of primary follicles. The strongest staining of AMH is observed in pre-antral and small antral follicles. AMH is found in growing follicles until they become dominant [11]. Thus AMH is a direct ovarian reserve marker representing different stages of growing follicles. AMH is undetectable after menopause and it does not seem to be regulated by FSH. Since cyclic variation of AMH is minimal, this test can be done at any time of the cycle. For evaluation of ovarian reserve, AMH seems to be more sensitive than other ovarian markers[12]. Low AMH value predicts poor ovarian response to ovarian stimulation.

However, AMH measurement is very expensive and not readily available in the developing countries. Adding AMH to the hormones to be evaluated for the initial investigation and for assessing ovarian reserve will make the total bill exorbitantly high thus making use of alternatives like

antral follicular count (AFC) using a transvaginal ultrasonography. AFC is a direct ovarian reserve marker. Antral follicles of 2-6mm are more predictive of the ovarian response than those measurements 7-10mm[13]. In general, the low range for AFC is comprised between 3 and 10 follicles. Low AFC indicates low ovarian reserve.

Measurement of basal estradiol in addition to FSH might improve the ability to predict fertility potential compared with basal FSH. Cycle day 3 E2 levels of less than 80pg/ml with normal FSH levels in women aged 38-42 years give a good prognosis of successful treatment. Some ART specialist are of the opinion to assess LH at day 2 and that if LH is high the patient may have to be commenced on oral contraceptive pills for one or two months to bring down the LH (LH > 3.5IU/ml) to prevent premature LH surge.

Serum inhibin B measurement has been used as an indicator of ovarian reserve. It is a glycoprotein produced by growing follicles in the early and mid-follicular phase. However, due to its lack of accuracy, it is not done routinely anymore [10].

Serum progesterone levels can be measured in patients being worked up for IUI. To confirm ovulation, serum progesterone levels are measured at mid- luteal phase or 21st day of a 28-day cycle. Serum progesterone levels greater than 4ng/ml is indicative of ovulation.

## OTHER ENDOCRINE TESTS

Hormonal disturbances impairing hypothalamic - ovarian axis such as thyroid dysfunction or hyperprolactinaemia should be evaluated. Serum thyroid stimulating hormone (TSH) and prolactin determinations can identify thyroid disorders and / or hyperprolactinaemia that may require a specific treatment. Clinical hyperandrogenism should be confirmed by serum androgen measurements including serum free testosterone, delta- 4-androstenedione, or dehydroepiandrosterone sulphate, biological hyperandrogenism requires further investigation to rule out the presence of non-classical congenital hyperplasia, crushing syndrome or androgen- producing tumor.

## PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

Preimplantation genetic diagnosis (PGD) is a type of embryo screening performed prior to implantation. It requires IVF to obtain the embryo or oocytes for evaluation. The aim of PGD is to ensure that the baby will be free from a specific inherited genetic defect that will give rise to a specific serious disease. The procedure would usually be used to screen for a specific disorder in couples with a high risk of transmitting an inherited condition (determined by previous pregnancies with serious genetic conditions or family history).

Different categories of diseases screened for by PGD include:

Autosomal dominant: Huntington's disease, Charcot-Marie-Tooth.

Autosomal recessive: Sickle cell haemoglobinopathies.

X linked: Fragile X, Haemophilia A, Duchene's

Chromosomal structural aberration.

Other forms of Preimplantation diagnostic procedures are

1. Preimplantation genetic screening (PGS) which screen embryos for aneuploidies (offered to patients who are undergoing IVF with advanced maternal age, history of recurrent miscarriages, family history of chromosomal problems/several unsuccessful IVF cycles).
2. Preimplantation tissue typing (PTT). This is similar to PGD and ensures that the child is free from the disease and is a tissue match for it's older affected sibling, hence the name Saviour siblings.

Ethical issues have arisen from the new advances offered by PGD, PGS and PTT. These and controversial aspects of PGD are treated in greater details in the section of this text on Legal & ethical issues in the management of infertility and assisted conception in Africa.

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