Male Subfertility -

2: Laboratory Investigations

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Several parameters can be used to evaluate the male partner of an infertile marriage, but the ultimate proof of fertility is a successful conception. Investigations performed to assess male fertility include tests to identify normal spermatogenesis and accessory sex gland function, followed by tests to assess the hormonal status of the patient. Other investigations may be further required to fully establish the diagnosis and initiate correct management.

Evaluation of the Ejaculate

The evaluation of the ejaculate is a very important part of the physical examination of a subfertile male. A semen analysis is relatively simple and several important conclusions can be obtained from the results. Techniques for the collection of the ejaculate include masturbation, condom, withdrawal, postcoital and prostatic massage samples. Masturbation is the only acceptable method for a thorough semen analysis. Provided attention is taken in the collection and transport of the specimen, reliable results are generally obtained. The length of abstinence before ejaculation causes significant changes in semen quality. The optimal period of continance is presently still subject to debate, but a period of abstinence of about four days is generally recommended. Collection of the specimen should be in a wide-necked ceramic or glass container with a screw top. This type of container prevents spillage during collection or transportation. Plastic containers may effect the motility of spermatozoa. After ejaculation, the specimen is kept at room temperature, and should reach the laboratory as soon after collection as possible. The analysis should be performed within 1 hour after collection of the ejaculate. The results of any specimen that arrives in the laboratory more than 60 minutes after ejaculation should be questioned. If more than 2 hours have elapsed, definite changes will have occurred. When a patient produces a completely normal semen sample, it is generally not necessary to examine additional specimens. However, if the sample is of a borderline quality or abnormal, further samples should be obtained before a diagnosis can be reached. Three separate analysis of semen samples obtained three to four weeks apart allows more definite information.

The most important parameters relating to fertility in the semen analysis are the sperm concentration and sperm motility. Other parameters are useful to identity disease of the accessory sex organs. Normal data for human semen parameters is given in Table 2:1. Abnormal semen analysis results may suggest further investigation of the ejaculate. Splitting the ejaculate in three portions allows for partial separation of the sex gland secretions. At the time of ejaculation, the bulbourethral glands secrete their fluids first lubricating the urethra. This is followed by prostatic secretions and spermatozoa mixed with epididymal and ampullary fluids, whereas the seminal vesicle secretions are ejaculated last. Thus the first fraction (volume 0.64±0.39 ml) has the highest sperm concentration ($203.4\pm173\times10^6$/ml) and lowest fructose content (132±87 mg/ml). It is also low in prostaglandins. The third fraction (volume 1.4±0.9 ml) has the lowest sperm concentration ($36.6\pm25\times10^6$/ml) and highest fructose content (414±240 mg/ml). Prostaglandin level is high. Prostatic secretion for biochemical or bacteriological analysis may be obtained by prostatic massage. Normal values for the biochemical secretions of the prostate are:

Acid phosphatase	$20\text{-}60\times10^3$ IU/ml
Citric acid	200-800 mg/100 ml
Zinc	80 - 250 µg/ml

Further examination of the ejaculate may be indicated. These may include bacteriological examintion to identify a genital tract infection, or a spermagglutination test to check for the presence of antisperm antibodies. Spermatozoon morphology may be further studied using specific staining procedures or electron microscopy. Biochemical tests can help establish the enzymatic functions of the spermatozoa to penetrate the ovum's zone pellucida during the fertilization process. Similar information can be obtained by using the Hamster ovocyte penetration (HOP) test.

The laboratory analysis or semen while being a useful investigation to assess male subfertility has the disadvantage that the tests alone cannot assess spermatozoa - cervical mucus activity. The in vivo postcoital test supplements the laboratory studies. The microscopic examination of postcoital cervical mucus has been performed for over 100 years. There are numerous reports as to the method of collection, time interval between coitus and examination, days of abstinence prior to testing, etc. It would appear that the test is best performed at midcycle two days prior to the shift in the basal body temperature, after two

TABLE 2:1 - **Normal Data for Human Semen Parameters**

PARAMETER	NORMAL DATUM	REMARKS
Coagulum	Present in fresh semen	Absence suggests absence or blockage of seminal vesicles
Liquefaction time of coagulum	5 - 20 min	>1 hr suggests poor prostatic activity
Odour	Similar to chestnut or carob tree flowers	Absent with impaired prostatic activity
Colour	Translucent to whitish grey; yellowish after long secual abstinence	Hemotospermia (red); leueospermia (white or yellow)
Viscosity	Fairly viscous, length semen thread 3 - 5 cm	High viscosity related to poor prostatic activity
Volume	1 - 6 ml averaging 3.5 ml	Hypospermia (<2 ml) suggests impairment of accessory sex gland function or endocrine hypogonadism. Hyperspermia (>6 ml) suggest overproduction by accessory sex glands
pH	7.2 - 7.8	>8.0 suggests acute inflammatory disease of accessory glands or epidydymis 7.2 suggest chronic disease of these organs
Percent motile spermatozoa	at 37 C 70% 1 hr after ejeculation; 60% 3 hrs after; half original motility 7 hrs after ejaculation.	Asthenospermia (<50% motility suggests sperm aging, infectious disease or presence of sperm antibodies. May be associated with agglutination (clumps of 4 or more spermatozoa)
Percent motile spermatozoa with forward progression	75% of motile spermatozoa	
Sperm viability	See percent motile spermatozoa	%immotile sperm>% dead sperms suggests presence of immobilizing agents or lack of nutrients for movement
Sperm concentration Sperm count	50 - 150 x 10^6 /ml averaging 90 x 10^6 /ml 100 - 500 x 10^6 averaging 300 x 10^6	Reflects sperm production, transport and function of accessary glands. Ploysperma: >250 x 10^6 /ml Oligospermia: <20 x 10^6 /ml Azoospermia: complete absence
Percent abnormal sperm forms	30 - 40% including abnormalities of the sperm head, sperm tail and spermatozoa precursors	Teratozoospermia (40%) suggests abnormality of spermatogenesis from debilitating illness, drugs, varicocoele, allergic reactions, hormonal, etc. allergic
Acid phosphatase(lu x 10^2 /ml) Fructose (mg/ml) Zinc (ug/ml) Citric acid (mg/ml) Proteins (mg/ml) Prostaglandins (mg/ml) A - B group E F 19-OH A-B group Glycerylphosphorylcholine (umole/ml)	88 - 979 (408) 0.7 - 5.0 (3.0) 25 - 424 (197) 1.8 - 8.4 (5.1) 21 - 66 secreted by seminal vesicle 50 53 8 200 .0.85	secreted by prostate secreted by seminal vesicle secreted by prostate secreted by prostate secreted by seminal vesicle secreted by epididymis
White cell counts	3 - 4 WBC/hpf 400x	6 - 10 WBC/hpf suggest genital tract infection; >10 WBC/hpf constitute significant pyosemia.

Table 2:2 - **Evaluation of the Post-Coital Test (assess 5 high power fields).**

Spinnbarkeit	absent	<3 cm	3—6 cm	6—9 cm	>9 cm
Crystalization	amorphous	linear	2 branching	fern-like	—
Viscosity	incresed	moderate	decreased	—	—
Appearance	turbid	turbid with clear areas	clear with flakes	clear	—
Cellularity	15+ cells/hpf	10—15	5—9	—5	—
Spermatozoa	negative absent motion	negative immobile	1—6/hpf forward motion	6—20/hpf forward motion	20+/hpf forward motion

Table 2:3 - **Hormone Levels in Male Infertility (N: normal:↓ decreased;↑ increased)**

CLASSIFICATION	*LH*	*FSH*	*TESTOSTERONE*	*OESTROGEN*
Primary testicular failure	↑	↑	***N or*↓**	↓
Hypothalamic — pituitary disease	↓	↓	↓	↓
Increased androgens	N or↓	N or↓	|	-
Increased oestrogens	N or↓	N or↓	↓	↑
Thyroid disease	N	N	N	—

Normal Serum values for male:LH (2nd IRP-HMG): up to 25 mIU/ml
FSH (2nd IRP-HMG): up to 20 mIU/ml
Testosterone (18–50 yrs): 14–42 nmol/l
Oestradiol: up to 0.3 nmol/l
Progesterone: up to 0.4 nmol/l
Prolactin (1st IRP 75/504): up to 700 mU/l

days of abstinence with a time interval from coitus to examination of two hours (rule of two's). The patient is asked to stay quietly in bed for some time with her knees bent and legs slightly spread out. From the information available in the literature, it is difficult to establish standards for defining normal post-coital tests. It would appear that a descriptive classification is the best terminology to describe the test (Table 2:2). The test allows the assessment of cervical mucus, the number of spermatozoa in the mucus and their motility pattern. A fractional postcoital test allows assessment of the *filtering* phenomenon which takes place when sperm penetrates into the column of cervical mucus. Abnormalities in motility and cervical mucus penetration noted by the in vivo postcoital test can be further investigated by in vitro sperm penetration tests (eg. cross-matching capillary tube test) and sperm agglutination tests (eg. Microtray agglutination test). The Cross-matching capillary test enables in vitro assessment of sperm penetration in cervical mucus and compares this to sperm penetration in donor mucus and donor sperm penetration in the patient's mucus. Immune reactions against spermatozoa as a cause of subfertility in the male or female partner are assessed by the Microtray agglutination test. Serum antibodies against sperm may belong to one or all of the three major immunoglobulins: IgG, IgA and IgM. The serum antibody level is regarded as significant if agglutination occurs at 1:32 or greater dilutions. In sera of man, the head-to-head agglutinins are largely of the IgM class, while the head-to-tail agglutinins of the IgG class.

Endocrine Evaluation

The testis is an endocrine organ whose function is closely linked to the hypothalamic - pituitary axis. Testicular failure may be of primary testicular origin when it is associated with castration with changes in the hypophysis and an increase in the gonadotrophin levels in the presence of a low serum testosterone. When secondary hypogonadism results from a hypothalamic - pituitary disturbance, the gonadotrophin levels are diminished along with the testosterone levels. Steroid hormone biosynthesis also takes place in the adrenal gland. Adrenal disease such as congenital androgenital syndrome may result in oligospermia or azoospermia in the post-puberty. In this situation, the testosterone level is elevated in the presence of normal or depressed gonadotrophin levels. Endogenous or exogenous hyperoestrogenism may also result in azoospermia with low testosterone levels and normal or decreased gonadotrophins. Prolactin plays some role in spermatogenesis and androgenic testicular function. Hyperprolactinaemia may be associated with hypogonadism. Radio-immunoassay of serum testosterone, oestradiol, FSH, LH and prolactin appear to be the more useful tests to assess the hypothalamic - pituitary - testicular axis (Table 2:3). Both hyper- and hypothyroidism have been implicated in male subfertility. Thyroid function tests would exclude these disorders. Blood glucose levels and a glucose tolerance test should be performed in patients with secondary organic impotence to rule out diabetes mellitus.

Diagnostic Radiology

Plain x-ray of the skull may show expansion and irregularity of the sella turcica in the presence of pituitary tumours causing secondary hypogonadism. More detailed information may be obtained from computerized axial tomography scans of the skull. X-ray examination of the bones around the big joints and the hand helps to establish the bone age which lags behind the chronological age in hypogonadism. Plain X-ray of the urogenital tract may reveal calcification in the seminal vesicles and the prostate. Congenital disorders of the Wolffian system are frequently associated with congenital anomalies of the urinary system. Intravenous pyelography helps to detect these associated anomalies. Ascending and descending cystourethrography may help diagnose a structure of the urethra which may be a cause of aspermia.

Injection of radio-opaque medium to visualize the seminal ducts has been employed to determine their patency in cases of male infertility. Vasoseminal vesiculography has been used to evaluate the vas deferens after vasectomy or prostatic surgery. It has also been used to differentiate between benign hypertrophy and carcinoma of the prostate and in detecting early metastatic lesions in the seminal vesicles. Varicography, the injection of radio-opaque dye into the spermatic vein, may help confirm the presence of a varicocele and to identify the best site of ligation of the spermatic vein during varicocelectomy.

Other Investigations

The incidence of varicocoele in patients with infertility is more than twice that in a random male population, and appears to cause disturbed spermatogenesis. Varicocoele should therefore be sought for in all patients presenting with subfertility. While clinical examination may help identify many cases of varicocoele, some cases may be more difficult to detect by clinical means alone. These patients may be identified by the use of the technique of scrotal thermography and the use of the Doppler stethoscope. B-mode ultrasonography has been used in the identification and diagnosis of intratesticular disorders. A characteristic echo pattern is obtained from the region of the rete testis but otherwise the testis remains echo-free. Hydrocoele, testicular abscess, and tumours also have characteristic patterns. The technique affords an accurate measurement of testicular thickness and may have the potential for revealing early testicular neoplasms.

Testicular dysfunction may be associated with abnormalities of the sex chromosomes so that 5 - 15% of infertile males have a chromosomal aberration and 15% have undetected translocations. Karyotyping of the infertile man would help identify these cases. Testicular biopsy is another important diagnostic method of male subfertility in cases of azoospermia in order to distinguish cases of obstruction in the duct system from cases of disorders of spermatogenesis.