

Male Factor

Etiology

- ◆ Central: Hypothalamic-pituitary disorders (GnRH, LH and FSH deficiency)
 1. Congenital GnRH deficiency (Kallmann's syndrome)
 - a. Hyposmia or anosmia in addition to gonadotropin deficiency. Abnormal development of the olfactory bulbs and tracts results in the decreased sense of smell.
 - b. The association between GnRH deficiency and anosmia appears to be related to the proximity of GnRH and olfactory neurons during embryologic development.
 - c. Other abnormalities associated with Kallman syndrome include cryptorchidism, cleft lip, cleft palate, and congenital deafness. Phenotypic appearance includes eunuchoid stature and sexual infantilism or incomplete sexual development.
 - d. Semen analyses to evaluate isolated gonadotropin deficiency reveal conditions ranging from azoospermia to severe oligoasthenospermia.
 2. Hemochromatosis
 3. Multiorgan genetic disorders e.g. Prader-Willi syndrome, Laurence-Moon-Beidl syndrome, familial cerebral ataxia
 4. Pituitary and hypothalamic tumors e.g. macroadenoma, craniopharyngioma
 5. Infiltrative disorders e.g. sarcoidosis, histiocytosis, tuberculosis, fungal infections
 6. Trauma, postsurgical, postirradiation
 7. Vascular: infarction, aneurysm
 8. Hormonal: hyperprolactinemia, androgen excess, estrogen excess, cortisol excess, hypothyroidism
 9. Drugs: opioids and psychotropic drugs, GnRH agonists or antagonists
 10. Systemic disorders e.g. Chronic illnesses, Nutritional deficiencies, Obesity
- ◆ Gonadal disorders
 1. Klinefelter's syndrome (XXY) and its variants (XXY/XY; XXXY)
 - a. Incidence: 1 in 500 newborns (i.e. relatively common).
 - b. Diagnosis:
 - (1) small, firm testes, with hyalinization of the seminiferous tubules, and azoospermia,
 - (2) gynecomastia.
 - (3) tall stature,
 - (4) normal male external genitalia,
 - (5) poorly developed facial hair.

- (6) Mental retardation, learning disabilities, and other social impairments may be present.
 - (7) There is an increased incidence of major and minor congenital anomalies.
 - (8) Variant and mosaic forms of Klinefelter syndrome exist and may be associated with testes of normal size and variable degrees of spermatogenesis.
2. Cryptorchidism
 3. Myotonic dystrophy
 4. Functional prepubertal castrate syndrome (congenital anorchia)
 5. Androgen insensitivity syndromes
 6. 5 alpha reductase (type 2) deficiency
 7. Y chromosome deletions
 8. Viral orchitis: mumps, echovirus, arbovirus
 9. Granulomatous orchitis: leprosy, tuberculosis
 10. Epididymo-orchitis: gonorrhea, chlamydia
 11. Drugs e.g. cytotoxic drugs, alkylating agents, alcohol, marijuana, antiandrogens, ketoconazole, spironolactone, histamine receptor antagonists
 12. Ionizing radiation
 13. Environmental toxins e.g. dibromochloropropane, carbon disulfide, cadmium, lead, mercury, environmental estrogens, phytoestrogens
 14. Hyperthermia
 15. Varicocele: dilatation of the pampiniform plexus of the scrotal veins. It is the most observed correctable cause of male infertility. Varicocele is present in 15% of the normal population, and in 25% to 40% of men with male factor infertility. It influences semen quality by increasing testicular temperature. The incidence of varicocele is much higher in male factor secondary infertility than in primary infertility, suggesting that the varicocele causes progressive impairment in spermatogenesis.
 16. Immunological, including polyglandular autoimmune disease
 17. Trauma, Torsion, Castration
 18. Systemic illness e.g. renal failure, hepatic cirrhosis, cancer, sickle cell disease, amyloidosis, vasculitis, coeliac disease
- ◆ Disorders of sperm transport (posttesticular)
1. Epididymal dysfunction: drugs, infection
 2. Abnormalities of the vas deferens:
 - a. Congenital bilateral absence of the vas deferens (CBAVD) can be diagnosed by the absence of fructose in the semen and confirmed by vasography. Many men with CBAVD are carriers of mutations in the gene for cystic fibrosis.
 - b. Ductal obstruction (Young's syndrome)
 - c. Infection
 - d. Surgery:

- (1) Previous vasectomy
- (2) Accidental ligation during inguinal surgery.
3. Ejaculatory duct obstruction: intraductal stones, stenosis, and intraprostatic cysts. Intraprostatic cysts causing obstruction of the ejaculatory duct may be of mullerian duct, wolffian duct, or prostatic origin.
4. Hypospadias, epispadias.
5. Sexual Dysfunction/Faulty Coital Technique:
 - a. Spinal Cord Injury: Men with spinal cord injury often experience impotence, difficulty with ejaculation, and reduction in testosterone production and spermatogenesis.
 - b. Autonomic dysfunction
 - c. Impotence
 - d. Retrograde Ejaculation
 - (1) Is caused by
 - (a) bladder neck surgery
 - (b) transurethral resection of the prostate
 - (c) Injury to the lumbar sympathetic nerves e.g. retroperitoneal surgery
 - (d) Diabetic neuropathy
 - (e) multiple sclerosis
 - (2) Diagnosis is suggested by low semen volume (hypospermia) and is confirmed by a high number of sperm in the urine after ejaculation.
 - e. Premature ejaculation
 - f. Spermicidal lubricants
- ◆ Idiopathic oligoasthenospermia

Diagnosis

Semen Analysis

- ◆ Semen analysis is the primary screening test for male factor infertility.
- ◆ If the first semen analysis is normal, there is generally no need for a repeat analysis. If an abnormal semen sample is obtained, an additional semen sample should be examined after an interval of 6-12 weeks.
- ◆ Procedure
 1. Before obtaining the semen specimen, the patient is instructed to abstain from ejaculation for a minimum of 48 hours and no longer than 7 days.
 2. The method of choice for collection is masturbation with avoidance of potentially spermicidal lubricants.

3. The specimen should be collected in a clean container and brought to the laboratory within 1 to 2 hours.
 4. Semen may be evaluated by an experienced technician or by semiautomated computers.
- ◆ Normal ranges for the major semen parameters are:
1. Volume (ml): 2-6
 2. Sperm density ($\times 10^6/\text{ml}$): >20
 3. Total number of spermatozoa ($\times 10^6/\text{ejaculate}$): >80
 4. Live spermatozoa %: ≥ 50
 5. Motile spermatozoa %: >50
 - a. 1 hour after ejaculation: ≥ 70
 - b. 3 hours after ejaculation: ≥ 60
 - c. 4 hours after ejaculation: >50
 6. Progressive motility score: 3-4
 - a. 0 signifies no motility
 - b. 1 denotes sluggish or nonprogressive movement
 - c. 2 refers to sperm moving with a slow, meandering forward progression
 - d. 3 signifies sperm moving in a reasonably straight line with moderate speed
 - e. 4 indicates sperm moving in a straight line with high speed.
 7. normal morphology %: ≥ 60
 - a. defective heads %: <35
 - b. defective midpiece %: ≤ 20
 - c. defective tails %: ≤ 20
 8. immature forms %: <4
 9. liquefaction: complete in 10-30 minutes
 10. leukocyte count/ μL : ≤ 2000
 11. pH: 7.2-7.8
 12. Fructose mg/dl: 150-600
- ◆ Abnormal parameters
1. Aspermia: No ejaculate
 2. Hypospermia: ejaculate volume <2 ml
 3. Azoospermia: No spermatozoa in the ejaculate
 4. Oligozoospermia: Sperm concentration $<20 \times 10^6/\text{ml}$
 5. Asthenozoospermia: $<50\%$ spermatozoa with forward progression (categories 3 and 4) or $<25\%$ spermatozoa with category 4 movement
 6. Teratozoospermia: $<30\%$ spermatozoa with normal morphology

7. Oligo-astheno-terato-zoospermia: Signifies disturbance of all three variables (combination of only two prefixes may also be used)
8. Lack of fructose and acidic pH suggest seminal vesicle dysfunction or ejaculatory duct obstruction.
9. Hyperviscosity (the absence of liquefaction) may result in relatively immobilized spermatozoa.

Testing Sperm Function

- ◆ Sperm penetration assay (SPA) (zona-free hamster egg penetration test),
 1. It is designed to assess the ability of sperm
 - a. To undergo capacitation and the acrosome reaction,
 - b. To fuse with and penetrate the egg membrane, and
 - c. To undergo chromosomal decondensation.
 2. The SPA does not test the ability of sperm to penetrate the zona pellucida.
 3. Sperm are washed free of seminal plasma and incubated in a protein-enriched medium that promotes capacitation. After incubation, motile sperm are separated and used to inseminate hamster eggs that have been treated with enzymes to remove the cumulus and the zona pellucida. Sperm from a donor of proven fertility is tested simultaneously as a positive control. Several hours after insemination, the hamster eggs are examined for sperm penetration. Penetration rates of less than 10% to 15% are considered abnormal.
- ◆ Hemizona test assesses the ability of spermatozoa to bind the zona pellucida.
- ◆ Tests to detect Acrosomal reaction
- ◆ Hypoosmotic swelling test measures the functional integrity of sperm membranes by placing specimens in hypoosmotic fluids.
- ◆ Sperm nucleus maturation can be determined by staining a sperm specimen with acidic aniline blue, acridine orange fluorescence, or sodium dodecyl sulfate decondensation.

Additional tests

- ◆ Antisperm Antibody tests: for patients with asthenospermia, sperm agglutination, or poor cervical mucus penetrability
- ◆ Microbiologic studies: if there is any suggestion of genital tract infections.
- ◆ Quantitation of leukocytes in semen: In the presence of excess white blood cells, sperm may fail to penetrate specially prepared hamster eggs or human eggs during in vitro fertilization (IVF). Leukocytes are difficult to distinguish from immature germ cells on routine semen analysis, but immunohistochemical staining using monoclonal antibody technology can determine whether these round cells are in fact white blood cells
- ◆ Reactive Oxygen Species (the superoxide anion, the hydroxyl radical, and the hypochlorite radical). Reactive oxygen species damage spermatozoa by attacking the sperm membrane and causing lipid peroxidation by a chain reaction mechanism.

- ◆ Radiology:
 1. transscrotal high-resolution color flow Doppler ultrasound: is the gold standard to diagnose varicocele
 2. Transrectal Ultrasound:
 - a. to confirm the presence of a varicocele
 - b. to evaluate complete or partial blockage of the ejaculatory ducts and seminal vesicles
 3. Vasography: indications are
 - a. absolute indications: Azoospermia, plus Complete spermatogenesis with many mature spermatids on testicular biopsy, plus At least one palpable vas.
 - b. Relative indications:
 - (1) Severe oligospermia with normal testicular biopsy
 - (2) High level of sperm-bound antibodies that may be due to obstruction
 - (3) Low semen volume and very poor sperm motility (partial ejaculatory duct obstruction)
- ◆ A karyotype for patients with azoospermia or severe oligospermia
- ◆ Testicular biopsy for patients with azoospermia or severe oligospermia
- ◆ Hormonal assay: for patients with azoospermia, oligospermia, or asthenospermia: serum testosterone, LH, FSH, TSH, prolactin

Table 2 Hormonal Status as a Function of Clinical Diagnosis

Status	FSH	LH	Testosterone
Normal men or obstruction	Normal	Normal	Normal
Isolated spermatogenic failure	↑	Normal	Normal
Testicular failure	↑	↑	Normal or ↓
Hypogonadotropic hypogonadism	↓	↓	↓

Treatment

- ◆ Treatment of the cause
 1. obstructive azoospermia
 - a. Microsurgery
 - (1) Microsurgical vasovasostomy using the Multilayer Microdot Method
 - (2) Crossed Vasovasostomy. is indicated in:
 - (a) Unilateral inguinal obstruction of the vas deferens associated with an atrophic testis on the contralateral side. A crossover vasovasostomy should be performed to connect a healthy testis to the contralateral unobstructed vas.
 - (b) Obstruction or aplasia of the inguinal vas or ejaculatory duct on one side and epididymal obstruction on the contralateral side.
 - (3) Microsurgical vasoepididymostomy: End-to-side or End-to-end

- b. Sperm harvest procedures combined with assisted reproduction and ICSI.
 2. Varicocele: high spermatic vein ligation leads to a fourfold increase in fecundability. Repair of a varicocele should be considered if male factor infertility is documented, female infertility evaluation results are normal, and a varicocele is clinically detected on physical examination.
 3. Hypogonadotropic hypogonadism is a rare cause of male infertility that usually can be successfully treated. Treatment goals include the stimulation of secondary sexual characteristics and of normal spermatogenesis.
 - a. hCG and hMG.
 - b. pulsatile GnRH.
 4. Spinal cord injury: electroejaculation can be used to obtain semen, but the semen often has abnormalities of sperm motility. As a result, electroejaculation may be combined with intrauterine insemination or assisted reproduction.
 5. retrograde ejaculation:
 - a. Medical treatment with pseudoephedrine 60 mg four times a day is recommended to stimulate closure of the bladder neck sphincter.
 - b. An alternative treatment is to collect and process sperm from a urine specimen immediately after ejaculation for intrauterine insemination or assisted reproduction. Urine should be alkalinized for optimal sperm function with 650-mg sodium bicarbonate tablets four times a day.
 6. Hypothyroidism: Normal fertility is often restored with thyroxine replacement.
 7. Klinefelter syndrome:
 - a. Therapeutic donor insemination (ethical debate).
 - b. The role of assisted reproduction in men with gonadal failure has not been defined, but it appears to be associated with a poor prognosis. The successful extraction of viable sperm from testicular biopsy specimens among men with Klinefelter syndrome or other causes of gonadal failure have been reported. Successful fertilization has occurred with ICSI, and subsequent pregnancies have been normal.
- ◆ Empiric therapy of Idiopathic oligoasthenospermia:
1. Empiric medical therapy
 - a. Clomiphene Citrate and Tamoxifen increase the hypothalamic secretion of gonadotropin-releasing hormone and therefore the pituitary secretion of LH and FSH, as well as the testicular production of testosterone.
 - (1) Clomiphene citrate: 25 mg daily given cyclically for 25 days with a 5-day rest period is the standard recommended treatment.
 - (2) Tamoxifen 10 to 20 mg daily
 - b. Human Chorionic Gonadotropin is used empirically in the treatment of oligospermia. Its intrinsic LH-like activity may increase the intratesticular concentration of testosterone that is thought to be deficient in patients.
 - c. Follicle-Stimulating Hormone is used in the treatment of men participating in an IVF treatment program who had severe seminal abnormalities or fertilization failure.

- d. Testolactone and Mesterolone
 - (1) Testolactone, an aromatase inhibitor, prevents the conversion of testosterone to estradiol and thereby minimizes the negative effect of the latter on spermatogenesis.
 - (2) Mesterolone is a synthetic androgen used to treat idiopathic male infertility.
 - e. Gn-RH: administered in a pulsatile fashion in programmable portable mini pumps.
 - f. Testosterone Rebound therapy involves large doses of exogenous testosterone that are administered parenterally to suppress the activity of the patient's pituitary gland. This, in turn, reduces the intratesticular level of testosterone to systemic levels from the usual level, which is 50 times the serum concentration. The androgen therapy is then stopped in the hope that the system will rebound and improved spermatogenesis will result.
 - g. Kallikrein causes the release of kinins in male and female genital secretions. kinins cause vasodilation and enhance sperm motility.
 - h. Pentoxifylline (is in the same pharmacologic class as caffeine and theophylline) inhibits phosphodiesterase thus generating an increase in intracellular cAMP, which, in turn, increases intracellular ATP production. This increase in ATP enhances sperm motility.
 - i. Antioxidants: Vitamin E is an effective antioxidant that improves sperm-oocyte fusion in vitro. Platelet-activating factor, lyso-PAF, and lysophosphatidyl choline are noted to increase sperm motility in vitro and may act as scavengers of reactive oxygen species.
2. Assisted reproduction: steps are based on the severity of the male factor.
- a. Mild Male Factor Infertility
 - (1) Expectant management (three cycles)
 - (2) Intrauterine insemination (three cycles).

Mild:
Sperm density (10⁶/mL): 15-20
Motility (%): 40-50
Morphology (% Normal): 30-40

 - (a) Patients with mild male factor infertility are considered better candidates for IUI. Treatment of moderate to severe male factor infertility with IUI is associated with minimal improvement of fecundability.
 - (b) The reported fecundability rate of IUI among patients with male factor infertility ranges from 0.02 to 0.21.
 - (c) Ovarian stimulation with IUI appears to increase fecundability among patients with male factor infertility.
 - (3) In vitro fertilization with or without intracytoplasmic sperm injection (six-cycle maximum)
 - b. Moderate Male Factor Infertility
 - (1) Intrauterine insemination with or without ovulation induction (three cycles)

Moderate:
Sperm density (10⁶/mL): 10-15
Motility (%): 20-40
Morphology (% Normal): 10-30

- (2) In vitro fertilization with or without intracytoplasmic sperm injection (six-cycle maximum)
- c. Severe Male Factor Infertility
- (1) In vitro fertilization with intracytoplasmic sperm injection (six-cycle maximum)
- (2) Consider therapeutic donor inseminations (ethical debate)

Severe:
 Sperm density (10⁶/mL): <10
 Motility (%): <20
 Morphology (% Normal): <10

Unexplained Infertility

- ◆ Perhaps the most frustrated patients, and the most frustrating to take care of, are couples with unexplained infertility.
- ◆ Unexplained infertility: occurring in any couple who has failed to establish a pregnancy despite an evaluation that uncovers no obvious reason for infertility or after correction of the factor(s) identified as responsible for the infertility.
- ◆ The incidence of unexplained infertility appears to be decreasing, and it is estimated to be approximately 15%. Although unexplained infertility may represent the inability of diagnostic tests to identify a potential cause, alternatively it may represent a lack of understanding of some aspect of the reproductive process.
- ◆ Therapy: an empiric approach
 1. Expectant management with timed intercourse in female patients younger than 32 years old.
 2. Empiric clomiphene with or without intrauterine inseminations
 3. Intrauterine inseminations with or without clomiphene.
 4. Empiric gonadotropin therapy with intrauterine insemination.
 5. Assisted reproduction with in vitro fertilization or gamete intrafallopian tube transfer.

Assisted Reproductive Techniques

Artificial insemination

- ◆ Artificial insemination is the placement of washed sperm into the female reproductive tract. Placement can be intracervical, intrauterine, intraperitoneal, or intrafollicular. Most common is intrauterine insemination (IUI).
- ◆ Insemination can be with husband's sperm (AIH) or insemination with donor sperm (AID).
- ◆ Indications for IUI include:
 1. Cervical factor infertility
 2. Male factor infertility
 3. Endometriosis-associated infertility
 4. Unexplained infertility
- ◆ Procedure:
 1. Intrauterine insemination should be closely timed with ovulation. Ovulation prediction kits detect the luteinizing hormone (LH) surge which precedes ovulation by 12–36 hours. Also, intrauterine insemination is performed 36 hours after hCG injection.
 2. Sperm are washed to reduce antigenicity.
 3. The volume of inseminate that can be transferred into the uterus is 0.25 to 0.5 mL. Small amounts are used to avoid cramping and flushing the oocyte out of the tube. The volume is also limited by space within the uterus.
 4. Intrauterine insemination is a clinic procedure. First, the position of the uterus is determined. A speculum is placed in the vagina and the cervix is visualized. The sample of washed sperm is placed into the uppermost portion of the uterine cavity using an insemination catheter with a disposable tuberculin syringe. Occasionally a tenaculum is needed on the anterior lip of the cervix to straighten the endocervical canal. The sample is injected slowly over 30–60 seconds.

Sperm retrieval techniques

- ◆ Epididymal spermatozoa retrieval
 1. The main indication for epididymal sperm retrieval is obstructive azoospermia, caused by different disorders:
 - a. Congenital bilateral absence of VD (CBAVD)
 - b. Cystic fibrosis
 - c. Vasectomy or failed vasectomy reversal
 - d. Inoperable ejaculatory ducts or distal vasal obstruction
 - e. Postinflammatory obstructions (TB, gonorrhea, chlamydia)

- f. Radical cysto-prostatectomy
 2. In cases of nonobstructive azoospermia, epididymal aspiration should not be attempted because the epididymal lumen is collapsed and spermatozoa cannot be retrieved.
 3. two methods to obtain epididymal spermatozoa
 - a. Microsurgical epididymal sperm aspiration (MESA):
 - (1) General or regional anesthesia.
 - (2) Scrotal longitudinal incision is carried down to the tunica vaginalis.
 - (3) After exposing the epididymis, at a magnification varying between $\times 6$ and $\times 40$, a tubule of the caput epididymis is longitudinally incised.
 - (4) The outflowing fluid is collected in a 22 Medicut attached to a tuberculin syringe, prefilled with small aliquots of medium to avoid the risk of drying out the sample.
 - (5) Each aspirate is handed to the biologist to assess for quantity and quality of sperm. This information will aid the surgeon on whether to continue the aspiration in the same place or move to a different segment.
 - (6) At times, it is necessary to perform aspirations from the vasa efferentia. If no sperm are found in the vasa efferentia, testicular sperm retrieval should follow. Epididymal sperm retrieval is completed once motile (even twitching) spermatozoa are found.
 - b. Percutaneous epididymal sperm aspiration (PESA):
 - (1) The patient requires conscious sedation and/or only spermatic cord block (0.5% Marcaine).
 - (2) The testis is immobilized with one hand while the aspiration is carried out with a butterfly needle connected to a 20-cc plastic syringe, inserted through the scrotum directly into the proximal caput of the epididymis.
 - (3) An assistant is required to pull the syringe plunger to create a negative pressure. If the tip of the needle is properly positioned, epididymal fluid will be seen flowing within the plastic tubing of the butterfly needle.
 - (4) In the laboratory, the needle is flushed four to five times in a Petri dish and a drop of this solution is examined under the microscope to check for motile sperm.
 - (5) The epididymal fluid may contain very few or many spermatozoa (sperm counts can fluctuate from few thousands up to 200 millions).
 - (6) Generally, two to three aspirates is all that is needed, but, if no sperm are found, six to eight aspirates from each side should be carried out before switching to testicular sperm retrieval.
- ◆ Testicular spermatozoa retrieval
1. The indications to obtain testicular spermatozoa are:
 - a. Nonobstructive azoospermia (maturation arrest, severe hypospermatogenesis, incomplete sertoli cell only)

- b. Obstructive azoospermia (rete testis blockage, no sperm in the epididymis, absent epididymis, extensive scarring)
 - c. Anejaculation (not responding to electroejaculation or vibrostimulation)
 - d. Complete terato/necrozoospermia
 - e. Complete sperm immobility
2. Currently, no clinical tests are able to predict reliably for the presence of sperm in the testes of patients with azoospermia in their ejaculate before resorting to the aspiration or biopsy. The use of molecular probes for meiosis provides a screening tool to identify differentiated germ cells. Absence of these markers indicates that no sperm can be retrieved from the testis.
 3. Testicular sperm can be retrieved by
 - a. Open or excisional biopsy TESE:
 - (1) For the open biopsy, a 1-cm transversal incision is carried through the tunica vaginalis down to the tunica albuginea, trying to obtain tissue from the midanterior surface of the testis.
 - (2) Gentle pressure is used to extrude testicular seminiferous tissue. The protruding seminiferous tubules are excised with scissors and transferred to a Petri dish containing 1 mL human tubal fluid (HTF)-buffered medium.
 - (3) A single biopsy is generally sufficient for the ICSI procedure and for freezing any excess testicular spermatozoa. However, in cases of incomplete Sertoli cells only or incomplete maturation arrest, up to three to four biopsies may be required.
 - (4) Micro-TESE: is a recent technique that uses of a microscope for selecting the seminiferous tubules to be excised. The assumption is that the seminiferous tubules containing spermatogenetic activity will appear more dilated.
 - b. Fine-needle TESA:
 - (1) The closed technique uses a transcutaneous aspiration by inserting a needle directly in the testicular parenchyma and using negative pressure.
 - (2) The number of passes through the testicular tissue may vary from 1 to 8 or 10.
 - (3) In the laboratory, the testicular tissue is finely minced in HTF-HEPES-buffered medium, and after centrifugation the pellet is suspended in culture media and examined for free testicular sperm.
 - (4) Testicular sperm show initially very low motility (flagellar twitching), which improves over time (10-12 h).
- ◆ Seminal tract washout (STW)
1. Indications:
 - a. ejaculatory duct incomplete obstructions, secondary to
 - (1) intraprostatic mullerian cysts between the two ejaculatory ducts,
 - (2) narrowing of the ejaculatory ducts after inflammation,
 - (3) functional emptying disturbances of the ampullo-vesicular tract due to
 - (a) diabetes

- (b) spinal cord injury
 - (c) extended retroperitoneal lymph node dissection
 - (d) idiopathic
- b. Anejaculation (absence of retrograde ejaculation)
- c. Postmortem collection
- 2. In these instances the technique of STW may be useful particularly when either electroejaculation or vibro-stimulation may fail.
- 3. The technique involves the cannulation of the vas deferens and the subsequent antegrade washing of the vas and collection of sperm from the bladder. The operating time is about 20 min, and the patient can go home in 1 h. At times, it is difficult to cannulate the vas and, thus, a hemi-vasotomy is required. The vas is then reapproximated by using microsurgical suture.
- ◆ Cryopreservation and thawing of retrieved spermatozoa
 1. Because the post-thaw recovery of motile epididymal sperm is optimized when the specimen is processed before cryopreservation, either washing or gradients for filtration are recommended.
 2. After sufficient sperm for ICSI have been set aside, the remainder of the specimen is pooled and processed.
 3. Washed specimens are first concentrated and then diluted 1:1 with freezing medium (TEST-yolk buffer with glycerol). Buffer media containing glycerol provide the most effective recovery of motile sperm after cryopreservation.
 4. For the thawing the cryo-vial is brought to room temperature or to 37 C, diluted with HTF-HEPES-buffered medium and washed once to remove the cryoprotectant.
 5. The pellet is then resuspended in a small aliquot of medium from which the motile or twitching epididymal sperm are isolated for the ICSI procedure.
 6. Similar procedures are used for testicular tissue, except the tissue must first be macerated and minced. Testicular tissue can be frozen either in stepwise fashion manually or by using the Planer Kryo III apparatus.
 7. Single sperm freezing technique: is required When the number of testicular spermatozoa is extremely small.
 - a. Sperm (up to five) and cryoprotectant (8% glycerol solution in phosphate-buffered saline supplemented with 3% human serum albumin) are inserted into liquid nitrogen.
 - b. The Cell-free human zona pellucida are loaded separately in 0.25-mL straws, exposed to nitrogen vapor overnight, and plunged the next day.

Assisted Reproduction and In Vitro Fertilization

- ◆ Rock and Menkin reported the first human IVF in 1944. However, the pioneering work of Edwards and Steptoe (1978) led to a birth after the first successful IVF-ET. Since the birth of Louise Brown, new advances have been developed.

- ◆ AR involves the direct handling and manipulation of the oocyte and sperm to enhance the probability of achieving a successful pregnancy.
- ◆ In Vitro Fertilization-Embryo Transfer
 1. Indications
 - a. tubal factor infertility
 - b. male factor infertility
 - c. endometriosis
 - d. Unexplained infertility
 - e. For all conditions that have not been successfully treated with other treatment strategies
 2. Patient Selection
 - a. Standard IVF is less successful for couples with severe male factor infertility if the total motile sperm count is less than 1.5 million
 - b. A woman's age is strongly predictive of IVF success or failure. This includes a decreased probability of conception and an increased probability of pregnancy loss with advancing maternal age. The association between maternal age and IVF outcome appears to be related to ovarian reserve. Most successful ART programs require some assessment of ovarian reserve (day 3 FSH level or CCCT) before IVF-ET to help counsel patients. In addition, most programs limit treatment with standard IVF-ET to women younger than 43 to 44 years of age. Patients older than 43 or patients with diminished ovarian reserve are poor candidates for standard IVF and should consider IVF with donor eggs
 3. Ovarian Stimulation
 - a. Although "natural" cycle IVF-ET avoids ovarian stimulation, it is limited by the harvest of only one to two oocytes. The natural cycle approach to IVF-ET is not done by most programs because of low rates of success. Similarly, ovarian stimulation with clomiphene alone is associated with the harvest of one to two oocytes and low rates of success
 - b. Controlled ovarian hyperstimulation (COH):
 - (1) Gonadotropin regimens may include hMG alone, FSH alone, or a combination of clomiphene citrate/hMG, clomiphene citrate/FSH, or hMG/FSH
 - (2) Optimal timing of oocyte retrieval is determined by follicular monitoring with ultrasonography and estradiol measurements. Follicular monitoring is also intended to minimize the risk for severe OHSS. The criteria for hCG administration during cycles of IVF-ET are significantly more aggressive in comparison with routine ovulation induction protocols combined with intercourse or intrauterine insemination. The criteria for hCG administration require estradiol levels greater than 600 pg/mL and at least two follicles larger than 20 mm. In contrast to ovarian stimulation with intercourse or intrauterine inseminations, estradiol levels greater than 2000 pg/mL are common
 - (3) GnRH agonist
 - (a) The addition of a GnRH agonist to gonadotropin stimulation offers several benefits

- (i) decreased premature ovulation,
 - (ii) decreased cycle cancellation,
 - (iii) increased number of oocytes,
 - (iv) increased number of embryos,
 - (v) increased number of successful pregnancies per cycle.
- (b) Protocols:
- (i) downregulation (long) protocols:
 - (a) mid-luteal initiation of GnRH agonist therapy followed by the addition of gonadotropin stimulation once ovarian suppression is achieved
 - (b) follicular-phase downregulation protocol: Follicular-phase downregulation reduces the likelihood of early pregnancy at the time of GnRH-agonist initiation. The disadvantage of this protocol includes the need to administer GnRH-agonist therapy for a greater number of days.
 - (ii) Flare (short) protocol: simultaneous initiation of GnRH-agonist and gonadotropin therapy during the early follicular phase.
 - (iii) Lower dosages (microdose) for women who respond poorly to conventional downregulation and discontinuation of the GnRH-agonist after ovarian suppression but before the initiation of gonadotropin stimulation.
- (c) type of GnRH analogue: daily subcutaneous formulations are preferred over depot intramuscular formulations.
- (4) GnRH antagonists
- (a) GnRH antagonists, cetrorelix and ganirelix, can be used to prevent premature LH surges in patients undergoing ovulation induction for IVF.
 - (b) GnRH antagonists are competitive inhibitors of GnRH. They bind to the GnRH receptor, blocking the release of bioreactive and immunoreactive LH, as well as the release of FSH. This suppression is observed within hours of administration and may last between 10 and 100 hours depending on the dose.
 - (c) The minimal effective dose for preventing a midcycle LH surge is 0.25 mg of either cetrorelix or ganirelix daily.
 - (d) A single 3-mg dose of the GnRH antagonist administered when the leading follicular diameter is greater than 14 mm will prevent an endogenous LH surge without the profound depletion of gonadotropins seen with the daily dose regimen.
- (e) Advantages:
- (i) Initiating gonadotropin stimulation at the beginning of the menstrual cycle, potentially reducing the dose and duration of gonadotropin treatment,
 - (ii) The ability to postpone or interrupt the LH surge, and

(iii) The ability to induce ovulation with native GnRH or a GnRH agonist i.e. suppression by GnRH antagonists may be overridden by exogenous administration of GnRH or a GnRH agonist.

(f) Disadvantages

(i) the fact that cycles may not be as easily programmed and

(ii) the need to replace exogenous LH if recombinant FSH is used.

(iii) Pregnancy rates are slightly lower than the rates using the agonist.

4. Oocyte Retrieval and Fertilization

a. Oocyte retrieval is scheduled 34 to 36 hours after hCG administration and uses a transvaginal ultrasound-guidance technique.

b. Timing of hCG administration should maximize oocyte maturation and minimize the risk for premature ovulation.

c. The procedure is performed with a long 18-gauge needle that has an echogenic tip to facilitate ultrasound visualization. Individual follicles are serially punctured, and follicular fluid is aspirated and transferred to an adjacent embryology laboratory for oocyte identification. The procedure takes 15 to 30 minutes. Anesthesia options include conscious sedation, spinal anesthesia, or light general anesthesia. The number of harvested oocytes is largely dependent on the number of follicles larger than 12 mm on the day of the retrieval.

d. Semen is collected the day of oocyte retrieval, usually by masturbation. Several sperm washing procedures have been described in combination with IVF-ET, including swim up and percoll gradient centrifugation techniques. The percoll gradient technique has become more popular for men with abnormal semen parameters because of increased fertilization rates. However, percoll has been routinely replaced with a synthetic equivalent, isolate, because of the theoretical concerns about viral contamination that could lead to Jakob-Creutzfeldt disease. Sperm are incubated in a protein-supplemented medium for approximately 4 hours to initiate the process of capacitation.

e. Oocytes are inseminated approximately 4 hours after their retrieval by coincubation with approximately 50,000 to 100,000 sperm per oocyte.

f. Approximately 12 to 18 hours after insemination, oocytes are examined for fertilization. Mature oocytes have a fertilization rate that ranges between 50% to 70%. A zygote with two pronuclei and two polar bodies is morphologic evidence of fertilization.

5. Embryo Culture and Transfer

a. The fertilized oocytes are placed in growth medium and typically not examined until the day of embryo transfer.

b. Assessment of embryo quality: by various morphologic characteristics such as number of blastomeres, degree of fragmentation, symmetry, granularity, vacuolation, membrane definition, and number of nuclei per blastomere.

c. The major goal of all ART programs is to maximize the chance for successful pregnancy and to minimize the risk for high-order multiple gestation. Unfortunately, live birth pregnancy and multiple gestation rates per cycle are both strongly dependent on the number and the quality of embryos transferred.

- d. The Society for Assisted Reproductive Technology (SART) suggests the transfer of no more than three embryos to women younger than 35 years of age and four embryos to women older than 35 years of age during an initial cycle of IVF-ET.
- e. Several programs have reported the routine transfer of two embryos to eliminate the risk for high-order multiple conception.
- f. Transfer of blastocyst-stage embryos 5 days after oocyte retrieval. Day 5 blastocyst transfer is associated with more strict and predictive criteria for embryo selection. Blastocyst transfer may be associated with superior live birth pregnancy rates and is usually limited to the transfer of one or two embryos. Thus, blastocyst transfer eliminates the risk for high-order multiple conception and prevents the transfer of unhealthy embryos that have discontinued the cell division required for normal development. The disadvantage of embryo transfer on day 5 is the possibility that none of the original cohort of fertilized oocytes will continue to develop in vitro for the entire 5 days.
- g. Embryo transfer procedure: The embryos are loaded into a flexible catheter with a small volume of culture medium. The catheter is gently passed through the cervical canal into the uterine cavity. The catheter is placed in the fundal region of the uterine cavity. Embryos are injected, and the catheter is slowly removed and passed to a member of the embryology staff. Then the catheter is carefully examined under the microscope for the possibility of a retained embryo. The Wallace catheter may be associated with an increased rate of success. Patients are discharged home 30 to 60 minutes after embryo transfer. It is recommended that they limit their activities for 24 to 48 hours.

6. Luteal Phase Support

- a. Luteal phase supplementation after oocyte retrieval improves pregnancy outcome. Relative progesterone deficiency may be related to
 - (1) disruption of the granulosa-luteal cells at the time of oocyte retrieval
 - (2) GnRH-agonist suppression of endogenous LH stimulation of the corpora lutea.
- b. The two most common methods of providing luteal phase support
 - (1) Progesterone: supplementation starts around the time of embryo transfer. When patients conceive, progesterone administration is typically continued until after the luteal-placental shift. Progesterone supplementation is the preferred method of luteal phase support because it is associated with a higher pregnancy rate and a lower rate of OHSS than hCG.
 - (a) a daily 50-mg intramuscular injection. This may cause severe myositis
 - (b) intravaginal progesterone suppositories
 - (c) oral micronized progesterone.
 - (2) HCG: 1500 to 10,000 IU intramuscularly once or more during the luteal phase.

◆ Tubal Transfer

- 1. Zygote intrafallopian transfer (ZIFT)
- 2. Pronuclear stage tubal transfer (PROST)
- 3. Tubal embryo transfer (TET)

4. Tubal transfer of cryopreserved embryos.
 5. Gamete Intrafallopian Transfer
 - a. Asch first described gamete intrafallopian transfer, or GIFT, in 1985.
 - b. GIFT requires general anesthesia and laparoscopy, which are performed immediately after transvaginal ultrasound-guided oocyte retrieval.
 - c. Four to six unfertilized oocytes are combined with washed sperm and transferred into the ampullary portion of the fallopian tube, usually under laparoscopic visualization with a flexible catheter placed through the fimbriated end of the fallopian tube.
 - d. Also, successful transfer of gametes into the fallopian tube through a transcervical approach with hysteroscopy or ultrasonography can be done.
 - e. GIFT requires a minimum of one easily accessible, normal-appearing, patent fallopian tube.
 - (1) The ideal candidates for GIFT are couples with
 - (a) unexplained infertility,
 - (b) early-stage endometriosis,
 - (c) and mild male factor.
 - (2) GIFT offers the theoretical advantage of placing oocytes and sperm in proximity in their natural or physiological tubal environment.
 - (3) The role of GIFT has been limited by ongoing improvements in embryo culture techniques and pregnancy outcome with IVF-ET.
 6. Tubal transfer techniques have the hypothetical advantage of combining the confirmation of fertilization with incubation in the tubal environment.
- ◆ Micromanipulation techniques
1. Partial zona drilling
 2. Subzonal sperm injection (SUZI).
 3. Intracytoplasmic Sperm Injection (ICSI)
 - a. It is the direct injection of a single sperm into the cytoplasm of the oocyte.
 - b. Success with ICSI is strongly dependent on
 - (1) the number and quality of oocytes.
 - (2) maternal age
 - (3) sperm motility (yet success with ICSI has been reported with immotile and immature sperm)
 - (4) gonadal reserve testing (FSH levels) in both partners.
 - c. Indications for ICSI continue to evolve and now include
 - (1) Absolute
 - (a) Severe oligospermia ($<10 \times 10^6$ /mL)
 - (b) Severe asthenospermia ($<10\%$ motility)

- (c) Epididymal or testicular sperm
- (d) Frozen thawed sperm with poor survival
- (e) Preimplantation genetic diagnosis
- (f) Absent fertilization in prior IVF cycle
- (2) Relative
 - (a) Sperm morphology <4%
 - (b) Antisperm antibodies
 - (c) Poor fertilization in prior IVF
- d. A major advance during the past decade is the combination of sperm harvesting techniques with ICSI.
- e. ICSI does not appear to improve the pregnancy rate with IVF if routinely offered to couples with normal semen parameters.
- f. Results of ICSI
 - (1) A fertilization rate of 70% was associated with live birth rate of 27% per embryo transfer.
 - (2) The rate of spontaneous pregnancy loss was 25%, and
 - (3) The rate of major congenital malformations was 3.3%.
- g. Genetic abnormalities associated with severe oligospermia or azoospermia may be transmitted to male offspring. So:
 - (1) Men with severe oligospermia or azoospermia who plan IVF with ICSI should consider cytogenetic analysis.
 - (2) second-trimester ultrasonography and amniocentesis should be offered for all ICSI-conceived pregnancies, regardless of maternal age.
- ◆ **Cyroeembryo Transfer (CET)**
 1. Cryopreservation of excess embryos enables indefinite storage for future transfer.
 2. Cyroeembryo transfer involves the timed transfer of thawed embryos. This can be accomplished during a natural cycle or after artificial preparation of the endometrial lining with a combination of GnRH-agonist downregulation plus estrogen and progesterone hormone replacement.
 3. Advantages of embryo cryopreservation:
 - a. the ability to avoid ovarian stimulation and oocyte retrieval.
 - b. the transfer of fewer embryos during the initial ovarian stimulation cycle. Transferring fewer embryos minimizes the risk for multiple gestation without compromising the overall pregnancy rate per retrieval.
 4. The probability of surviving the freeze-thaw process and successfully implanting is strongly dependent on
 - a. the quality of the embryo before freezing.
 - b. Cryoprotectant used: the live birth rate per embryo thawed with dimethyl sulfoxide (3.5%) is higher than with 1,2 propanediol (0.8%).

- c. stage of embryo development.
 - d. endometrial preparation.
5. Advancements in oocyte cryopreservation have generated concerns about spindle disruption that could cause chromosomal abnormalities.
- ◆ In Vitro Fertilization with Donor Oocytes
1. Treatment options available for women with ovarian failure or diminished ovarian reserve are limited. In the absence of IVF with donor oocytes, the remaining infertility treatments are associated with low probability of success.
 2. Initial indications for IVF with donor oocytes included
 - a. the various causes of premature ovarian failure, such as Turner syndrome, bilateral salpingo-oophorectomy, chemotherapy or radiation therapy, and idiopathic premature failure.
 - b. More recently, the indications for IVF with donor oocytes have been extended to
 - (1) perimenopausal and postmenopausal women,
 - (2) female carriers of genetic diseases, and
 - (3) infertile couples who cannot conceive after multiple cycles of traditional ART.
- ◆ Pregnancy Outcome of ART
1. The live birth rate per cycle initiated is 19%.
 2. The rate of early abortion is 20%.
 3. The rate of Ectopic pregnancy is 3%. It is more common among women with tubal factor infertility.
 4. The incidence of congenital malformation is 0.7% of all neonates.
- ◆ Major risks of ART
- a. OHSS: the evaluation and treatment of OHSS were described earlier in the section on gonadotropin therapy.
 - b. Multiple conception:
 - (1) During the past decade, the number of multiple pregnancies increased as a result of the increasing number of ART cycles.
 - (2) Rates of multiple conception with successful IVF cycles are 29.6% twins, 6.4% triplets, and 0.6% high-order multiple gestations.
 - (3) Multifetal pregnancy selective reduction:
 - (a) For Couples with triplets and high-order multiple gestations
 - (b) the procedure is performed with transabdominal ultrasound guidance at 12 to 13 weeks of gestation. Injecting potassium chloride into the fetal heart brings about rapid fetal demise.
 - (c) The risk for losing the entire pregnancy through spontaneous abortion after multifetal pregnancy reduction is estimated to be between 5% and 10%.
 - (d) Multifetal pregnancy reduction is associated with improved pregnancy outcome because of the decrease in early preterm deliveries.

(e) About 20% of couples offered selective reduction choose the procedure.

Advances in Assisted Reproduction

- ◆ Assisted hatching:
 1. Is a micromanipulation technique proposed to improve embryo implantation.
 2. The embryologist performs assisted hatching on the day of embryo transfer.
 3. It involves the thinning or disruption of the zona pellucida by enzymatic or mechanical micromanipulation, which may allow the embryo to expand and "hatch" through the zona pellucida, facilitating a favorable interaction between the embryo and the endometrium.
 4. It may improve the probability of successful implantation in embryos with thickened zona pellucida and in women with elevated FSH levels.
- ◆ Preimplantation genetic diagnosis:
 1. In the human, removal of one blastomere from a six- to eight-cell embryo to provide useful genetic information has been associated with successful implantation and pregnancy.
 2. Techniques such as fluorescent in situ hybridization and polymerase chain reaction are suitable for the genetic analyses because results can generally be obtained within less than 12 hours.
 3. Many genetic diseases can be evaluated with preimplantation genetic diagnosis and blastomere biopsy e.g. alpha-1 Antitrypsin deficiency, Cystic fibrosis, Tay-Sachs disease, Duchenne muscular dystrophy, Turner syndrome, Down syndrome, Hemophilia A, Fragile X syndrome, X-linked disorders
- ◆ Recently, the transfer of oocyte cytoplasm has been suggested as an alternative to IVF with donor oocytes for couples with severe oocyte abnormalities.
- ◆ In vitro oocyte maturation is another area of ongoing research, which, if properly developed, could be combined with the cryopreservation of ovarian biopsy specimens.