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REVIEW ARTICLE

ASSISTED REPRODUCTIVE TECHNOLOGY

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ABSTRACT

All the treatment or procedure that includes the handling of both human sperm and oocytes or embryos in vitro for the purpose of establishing a pregnancy in order to bypass some pathological obstacles in human reproduction is known as Assisted Reproductive Technology (ART). Now we must be approaching 1.5 million Assisted Reproductive Technology birth since the birth of the world's first in vitro fertilization baby, Louise Brown, in the United Kingdom. The infertility is caused by various reason and factors from either or both partners. Infertility affects worldwide by 8-15 percent of couples in general and defined as a disease of the reproductive system by the failure to achieve a clinical pregnancy after one year or more of regular unprotected sexual intercourse.

Key Words: *Assisted Reproductive Technology (ART), Clinical Pregnancy, Embryo Transfer (ET), In Vitro Fertilization (IVF).*

INTRODUCTION

Infertility clinically defined as a disease of the reproductive system by the failure to achieve a clinical pregnancy after one year or more of regular unprotected sexual intercourse.[1] Infertility is divided in two types primary infertility- a delay for a couple who have had no previous pregnancies and secondary infertility- a delay for a couple who have conceived previously, although the pregnancy may not have been successful (e.g. miscarriage, ectopic pregnancy). Clinically subfertility is used to describe women or couples who are not sterile but show decreased reproductive efficiency. Nearly 85-90 percent of healthy couples conceive within 1 year, most within six months.[2,3] It is generally accepted that one in four women are affected at sometimes.[4] Cycle fecundability is the probability that a cycle will result in pregnancy and fecundity is the probability that a cycle will result in a live birth. At first month of trying 30 percent of conception is most likely to occur. The chance then falls steadily to about 5 percent by the end of the first year of trying. Cumulative conception rates are around 75 percent after six months, 90 percent after a year and 95 percent at two year. The like hood of spontaneous conception is affected by cause of infertility, age, history of previous pregnancy, duration of infertility, timing of intercourse, BMI, personal habit[5,6] of smoking, caffeine & alcohol, drugs abuse, socioeconomical status. Infertility is caused by male, female, combined and unexplained reasons. The evaluation and treatment of infertility have changed dramatically since first IVF in 1978. ART includes methods for assisted fertilization by intracytoplasmic sperm injection (ICSI) using sperm isolated from the ejaculate or obtained by microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE), assisted embryo hatching, intrauterine insemination (IUI), oocytes pick up, embryo culture, embryo transfer and preimplantation genetic diagnosis (PGD). In most cases, IVF is used to help an infertile couple conceive their own biological child, but donor sperm, donor oocytes, and gestational surrogates play an important role in modern ART. Other forms of ART include tubal transfer of oocytes and sperm (gamete intrafallopian transfer; GIFT), zygotes (zygote intrafallopian transfer; ZIFT), or embryos (tubal embryo transfer; TET) via laparoscopy. An embryo Transfer (ET) is a final vital step in IVF treatment that decides overall success of ART Cycle[7] and clinical pregnancy rate[8,9] Embryo implantation and clinical pregnancy rates are highly influenced by anatomical and physiological factors of uterus[10,11] and embryo transfer technique.[7,12-17]

Epidemiology

Infertility affects worldwide by 8-15 percent of couples in general, with increased prevalence.[18] Global estimates in 2007 suggest that nearly 72.4 million couples experience fertility problem.[19] However, the exact number of prevalence is hard to estimate because of the appropriate survey method, criteria to establish the diagnosis of infertility, outcome measured that is pregnancy or live birth rate.

Causes of Infertility

The infertility is caused by various reason and factors from either or both partners. The abnormalities in the production of a competent oocyte, abnormalities in reproductive tract transport of the sperm, and embryo quality, abnormalities in the implantation process including early defect in embryo development and embryo endometrial reaction, abnormalities in

sperm production and other conditions including immunological factors that can affect multiple components of the process should be highly consider for the cause of infertility. Infertility causes factors are distributed[20] as female factors, male factor, unexplained, uncommon. At any time to the some extent, the prevalence of each cause of infertility varies with age.[21,22] The male factors and unexplained infertility are observed somewhat more often in older couples.[23,24] The distribution of causes of infertility also varies with the duration of infertility and the level of care.[25-27]

Female Factors Causing Infertility

Female fertility is defined as the capacity to conceive and produce offspring that affect by number of factors. The causes of female infertility include fallopian and ovarian factors, uterine and cervical factors, immunological and unexplained factors (including endometriosis with no evidence of tubal or peritoneal adhesions) table 1.[20] Hyperprolactinemia can occur at any age, and the prevalence varies from 0.4% in the normal adult population to as high as 9-17% in women with menstrual problems such as amenorrhea or polycystic ovarian syndrome.[28,29]

Overall, fertility rates are 4-8 percent lower in women aged 25-29 years, 15-19 percent lower in those aged 30-34, 26-46 percent lower in women aged 35-39, and as much as 95 percent lower for women aged 40-45 years.[21,30] Advanced age affect fertility by decreased in coital frequency[31] and in different other ways, such as age increase, increase incidence of abortion, greater chance of pelvic infection, leimyomata and endometriosis.

Table 1: Causes of Female Infertility and their distribution percentage

Ovulatory Disorders 40%
Anovulation Oligo-ovulation Endocrine disorder – Hypothalamic Pituitary damage Tumors - Prolactinoma , Acromegaly, Cushing's &Kallman's syndromes Hypogonadotropichypogonadism-Weight loss, psychological stress Thyroid disorders (Hypo), Adrenal disorders(CAH), Chemo- radiotherapy, Tuberculoma, Sarcoidosis, Syphilis Ovarian disorders PCOS, Premature ovarian failure –Turner syndrome(45X), Idiopathic Chemo- radiotherapy, Surgical removal of ovaries, Genetics or Autoimmune.
Tubal and Pelvic Factors 40%
Infection – Salpingitis, Hydrosalpinx, Tubal blockage or. Pelvic inflammatory disease :- Genital Tuberculosis, Chlamydia trachomatis, Gonorrhea, Peritubal adhesion Post pregnancy sepsis –History of septic abortion, Intrauterine adhesion. Intrauterine contraceptive devices Pelvic or tubal surgery – sterilization, appendectomy, ectopic pregnancy. Endometrium – Endometriosis, endometrial dysfunction Uterine Factors includes -congenital malformations, leiomyomas, and intrauterine adhesions, endometrial polyps Cervical Factor: Abnormalities of Sperm-Mucus Interaction
Uncommon factors 10%
Unexplained infertility 10% of cases

The most common causes of hypothalamic dysfunction are abnormalities in weight and body composition, eating disorders, stress, and strenuous exercise. The second most important factor for causing female infertility are tubal and pelvic factors such as infection e.g. salpingitis, pelvic inflammatory disease, hydrosalpinx, tubal blockage, genital tuberculosis,[32] chlamydia trachomatis, gonorrhea.[33,34] Intrauterine adhesions caused by post pregnancy sepsis, septic abortion, previous pelvic surgery are another burden for female fertility. Endometrium plays a vital role in infertility, endometriosis, adenomyosis, polyp, the others endometrial dysfunction, are major factor that affect infertility significantly. Prevalence of genital TB in the infertile population has been reported in both developed[33] and developing countries. The prevalence of uterine anomalies in infertile women and fertile women with normal reproductive outcomes is similar, approximately 2-4 percent.[35-40] Septate uterus is the anomaly most highly associated with reproductive failure and obstetrical complications, including first and second trimester miscarriage, preterm delivery, fetal malpresentation, intrauterine growth restriction, and infertility.[37,41]

Male Factors Causing Infertility

The male factors are responsible from 35 to 50 percent of subfertile couple[42] The main factors for male causing infertility are either abnormal semen analysis or sexual dysfunction, and are identifiable in approximately one in thirteen couples attempting to conceive[26,43-45] An etiological factors involved in male factor infertility are presented in table 2.

Table 2: List of etiological factors involved in male factor infertility.

Congenital factors Anorchia Cryptorchidism Congenital Absence of Vas Deferens Genetic abnormalities (caryotype anomalies including Klinefeltersyndrome (xxy); Y Chromosome mirodeletions; Kallmann syndrome, mutations in genes involved in Hypothalamus–pituitary–gonadal axis, Partial/Mild Androgen Insensitivity syndrome)
Acquired factors Testis trauma Testicular torsion Post-inflammatory forms (orchitis, epididymitis) Obstruction, subobstruction of proximal and/or distal urogenital tract Recurrent urogenital infections, prostatitis, prostatovesciculitis Exogenous factors (medications, cytotoxic drugs, irradiation, heat etc) Systemic diseases (liver cirrhosis, renal failure etc) Varicocele (depending on the grade) Surgeries that can damage vascularisation of the testes Erectile, ejaculatory dysfunction Acquired hypogonadotrophichypogonadism or endocrine factors
Idiopathic forms Unknown etiology (about 50%)

Semen analysis is of fundamental importance to diagnose and define the severity of the male factor. Semen volume, sperm motility, and the proportion of sperm morphology, but not sperm concentration, appear to decrease gradually as age increases[46,47] nomenclature to understand semen pathology[48] is given in table 3.

Table 3: Nomenclature related to pathological semen quality according to WHO

Oligozoospermia	Sperm concentration <15x106/ml;
Sperm concentration	total sperm number <39x106/ml
Asthenozoospermia	<32% progressively motile spermatozoa
Teratozoospermia	<4% morphologically normal spermatozoa
Oligo-asteno-teratozoospermia	Disturbance of all three parameters
Azoospermia	No spermatozoa in the ejaculate
Aspermia	No ejaculate
Leucospermia (leucocytospermia)	>1 x 106 ml leucocytes in the ejaculate
Cryptozoospermia	Spermatozoa absent from fresh preparation but observed a centrifuged pellet

Microscopic semen analysis should be performed and analyzed according to the WHO protocol[49,50] and result should be correlated with minimal standard given in table 4. Recently computer assisted semen analysis (CASA) has been developed to overcome the highly subjective nature of conventional analysis of sperm morphology and quality.[51] Reference ranges for sperm parameters have been recently updated and provided by 1999 and the last version of 2010 WHO manual.[49,52]

Table 4: WHO parameter percentile (95% CI)

	WHO 1999	WHO 2010
Semen volume	> 2.0	1.5 mL (1.4–1.7)
pH	> 7.2	> 7.2
Total sperm number	> 40	39 Million (33–46)
Sperm concentration(density)	> 20	15 Million/mL (12–16)
Vitality	> 75	58% Live (55–63)
Progressive motility	> 50	32% (31–34)
Total (progressive +non-progressive) motility	> 50	40% (38–42)
White blood cells	< 1.0	<1.0 x106 per ml
Morphological normal forms	N/A	4.0% (3.0–4.0)
Sperm antibodies	< 50	< 50 % of coated sperm

Unexplained Infertility

Unexplained infertility is a diagnosis of exclusion of female or male infertility. It is important to emphasize that all of the potential causes of unexplained infertility could co-exist with known causes for infertility, helping to explain why many couples with identified ovarian, male, uterine, or tubal infertility factors fail to achieve a successful pregnancy despite receiving proven effective treatments.[53-55]

Diagnosis of infertility

The clinical evaluation of infertile couple includes proper history taking and thoroughly evaluation of both partners with help of specific investigation like basic hormonal level, laparoscopy, HSG, hysteroscopy. Further assessment of ovulation, semen analysis and tubal patency evaluation comprise the initial basic work up for infertility diagnosis. There is clear evidence that preconception interventions for women with chronic diseases, poor reproductive history and adverse lifestyle behaviors may lead to improved fertility and pregnancy outcomes.[56-59] The age,[21,22,31] weight,[60,61] nutritional composition, smoking,[6,62] exercise,[63] alcohol,[5] caffeine,[64-66] illicit drugs, environmental pollutant, psychosocial stress,[67,68] immunization history, history of previous surgery, history of STI ,infectious disease, coital practice[31] are well established factors for infertility.

The relevant medical history and physical findings includes the[69] coital frequency, sexual dysfunction, symptoms of any systemic disease, signs of androgen excess, thyroid enlargement, nodule, or tenderness, pelvic or abdominal tenderness, organ enlargement, or mass, vaginal or cervical abnormality, secretions, or discharge. Worsening dysmenorrhea, new onset of dyspareunia, or physical findings of focal tenderness or cul-de-sac nodularity suggest endometriosis. Most basic investigation should be done to rule out the normal reproductive function are given in table 5. In patients with primary amenorrhea and an elevated FSH level, karyotype examination should be undertaken and may reveal a chromosomal abnormality. In women with secondary amenorrhea, an FSH level more than 30 IU/l indicates premature ovarian failure. Low FSH and estrogen levels are common in patients with hypogonadotropic hypogonadism. The elevated prolactin level may be associated with low FSH, LH and low/high TSH levels. Thyroid dysfunction is strongly associated with infertility. The TSH and free thyroxin levels should be measured in women with amenorrhea, oligomenorrhea, polymenorrhea or hyperprolactinemia. Neuroleptic drugs, antidepressants, hypotensive drugs (reserpine, methyldopa) and drugs for treating gastrointestinal symptoms may cause hyperprolactinemia. Elevated serum androgen levels may be found in women with ovarian dysfunction and hirsutism. Hyperandrogenemia may be of adrenal origin or ovarian origin.

Table 5: Diagnostic investigation of female infertility

Through history and physical examination Basal Body Temperature (BBT), Hysterosalpingogram (HSG) Hysterosalpingo-contrast sonography (HyCoSy) Laparoscopy and chromopertubation Fertiloscopy (transvaginalhydrolaparoscopy) The post-coital test (PCT) Radiological examination CT, MRI Ultrasonography – Specially TVS Ultrasound Follicular tracking To assess pelvic normality To assess ovulation Saline infusion sonohysterography	Routine vaginal exam Hormonal assays - P4, LH, FSH, PRL, TSH Hysteroscopy Endometrial biopsy Drug history –may cause hyperprolactinemia Falloposcopy Serum Androgen elevation. Genetic evaluation – Karyotypic analysis leukocyte karyotype analysis X-chromosome abnormalities

The diagnostic evaluations for male infertility are given in table 6. Male infertility is generally correctable[70] with minimal clinical surgical interventions[71] and genetic transmissible approach.

Table 6: Diagnosis of Male Infertility

History	Medical, surgical, developmental, genitourinary, reproductive, sexual
Physical examination	Body habitus, hair distribution, gynecomastia, hypospadias, volume & Consistency of testes, vas deferens, spermatic cord, varicocele, cryptorchidism.
Semen analysis	Ejaculating volume, pH, sperm concentration, total sperm number, sperm motility and sperm morphology.
Endocrine evaluation	Serum T, FSH, E, PRL
Genitourinary infection	UTI, Urethritis, prostatitis, epididymitis and orchitis
Ultrasonography	Scrotal ultrasonography, Transrectal ultrasonography (TRUS)
Post ejaculate urinalysis	To conform the retrograde ejaculation.
Genetic testing	Chromosomal specially Y- chromosome micro-deletion, Karyotypic analysis and Cystic fibrosis analysis, congenital absence of vas deferens patients.
Sperm DNA integrity testing	DNA fragmentation indexes (DFI) enable the cause of semen analysis showing occult male factors.
Antisperm antibody	Serum antisperm antibodies measure in case of asthenozoospermia.
History of drug uses	Sulfasalazine, methotrexate, nitrofurantoin, chemotherapy testosterone, gonadotropin releasing hormone analogues, cimetidine, spironolactone, β-blockers, antidepressants, phenothiazines, thiazide diuretics, metoclopramide, anabolic steroids, cannabis, heroin, cocaine, smoking, alcohol.
Other specialized test	Post coital test and Testicular biopsy

Treatment of Infertility

A diagnosis should allow consideration of medical and surgical therapies that permit natural conception. Natural fertility should always be the prime goal, both partners should be encouraged to attend each visit and discuss the physiology of conception and timing of intercourse including preconception counseling of both partners to optimize their health. Treatment should not be delay, because the probability for achieving a live birth without treatment decreases with increasing age and duration of infertility.[72-77] The treatment algorithm of infertility is given in Table 7.

Table 7: Treatment of infertility

Female infertility treatment: **Medical treatment** Ovulation Induction - Clomiphene Citrate, gonadotropin, insulin and metformin in PCOS cases, treatment of systematic illness if any. Hormonal suppression combination oral contraceptive pills, GnRH agonists, or danazol in endometriosis cases. Antibiotics in case of PID, Chlamydia trachomatis and Mycoplasma species and other infectious disease.
Surgical: **Hysteroscopy, laparoscopy, laparotomy** Polypectomy, congenital anomalies of vagina or uterus surgery, intrauterine adhesion surgery, sterilization reversal, tubal and peritoneal disease surgery. Laparoscopy allows careful assessment of the external architecture of the tubes, fimbria and identify tubal obstruction, pelvic adhesions, endometriosis, can be treated at the time of diagnosis. Laparoscopic salpingectomy or proximal tubal occlusion increases IVF success rates, wedge resection of the ovaries in PCOS. Laparoscopic chromopertubation and ovarian diathermy (or ovarian drilling) should consider in proximal tubal occlusion, hydrosalpinx tubal spasm, temporary mucous plugging, and underfilling of the tube. Laparoscopic electrocoagulation and resection of endometriosis.
Male infertility treatment: **Non-Assisted reproductive treatments** Effective medical interventions - sympathomimetic (imipramine, pseudoephedrine, ephedrine, phenylpropanolamine) Hypogonadism (testosterone, aromatase inhibitor, testolactone , anastrozole) Gonadotropin therapy, Immunosuppression and sperm antibodies, Withdrawal of drugs approaches of uncertain efficacy , Varicocele ligation/occlusion surgery, Cryptorchidism orchiopexy Varicocele repair, Lifestyle interventions, Antiestrogens, Antioxidants, Nutritional supplements, Historical and disproven treatments, Androgens, Antibiotics and anti-inflammatory drugs, Scrotal temperature, Empiric treatment (3-6 months) with either clomiphene citrate or tamoxifen commonly is offered to stimulate increased pituitary gonadotropin secretion and spermatogenesis in men with idiopathic subfertility.
Hypogonadotropic Hypogonadism In men with congenital hypogonadism and those with post pubertal onset who do not respond to treatment with hCG alone, normal spermatogenesis can be induced by combined treatment with hCG and hMG or pure FSH. A prolong treatment follow up and repeated semen analysis may reveal the success of treatment.
Retrograde Ejaculation Sympathomimetic, Alternatively, sperm can be recovered directly from the bladder after masturbation; for best results. The urine pH and osmolality (300-380 mOsm/L) must be carefully controlled by alkalinizing the urine (sodium bicarbonate 650 mg four times daily, beginning 1-2 days before collection) and managing fluid intake.
Assisted reproductive treatments Surgical sperm isolation for ICSI, Spermatogenic failure, Methods for surgical sperm isolation, Fine-needle aspiration (FNA)., Open testicular biopsy, microsurgical vasovasostomy or vasoepididymostomy, Ejaculatory duct obstruction – prostatic transurethral resection Cryopreservation of sperm after extraction.
Unexplained Infertility treatment Intrauterine insemination (IUI), Ovarian stimulation with clomiphene or gonadotropins and IUI, and IVF.
Uncommon Donor Oocyte, Donor insemination, Sperm, Surrogate, adoption

DISCUSSION

The prospect hold promise for rapid evolution in infertility world after the first pregnancy resulted from IVF in 1976, and was ectopic.[78] IVF has become a well-established treatment procedure since the birth of the first test tube baby in 1978.[79] IVF success depends on understanding of basic reproductive endocrinology and start with physiology of ovulation, spermatogenesis, fertilization, zygote and implantation into endometrium. Management of infertile couple started with counseling, treating all treatable cause of infertility and suitable planning of ART, at all the time natural conception should be encouraged. Embryo transfer is placing of embryos in the proper location within the uterus and technique is equally important as clinical and embryological characteristic of embryo in clinical pregnancy achievement in ART cycle.[8,9] Male infertility caused by low sperm counts, impaired motility, and poor morphology represent the main causes of failed fertilization in conventional IVF. To tackle this problem, several procedures of assisted fertilization based on micromanipulation of oocytes and spermatozoa have been established. These strategies have culminated in intracytoplasmic sperm injection (ICSI), in which a single spermatozoon is directly injected into the ooplasma. First human pregnancies and birth after ICSI reported in 1992.

Since then, the number of worldwide centers offering ICSI has increased tremendously. An ICSI represents the major breakthrough for male infertility treatment, preimplantation genetic diagnosis (PGD) would be inconceivable without the establishment of embryo biopsy procedures. Initial attempts to penetrate or open the zonapellucida were undertaken to assist oocyte fertilization[4,19,43] or increase embryo implantation by facilitating the hatching process.[80,81] This very close access to oocytes or embryos soon resulted in the removal of cellular material (polar bodies or blastomeres, respectively), which allowed genetic testing in this very early developmental stage.[82-85] As much as PGD relies on embryo biopsy, equally indispensable was the progress in molecular genetics leading to the development of diagnostic procedures at the single-cell level.[86-88] These procedures include gene amplification using polymerase chain reaction (PCR) for monogenic diseases and fluorescence in situ hybridization (FISH) for the evaluation of numeric and structural chromosome aberrations or for sex determination in cases of sex-linked diseases. PGD evaluation has increased clinically worldwide, more recently.[89] Aneuploidy screening (PGDAS) has been introduced in attempt to increase the implantation rate in older women, in couple with previous ART failure, and in couple with recurrent abortion.[90] Mapping of Y chromosome has been possible due to advances in molecular medicine. Testicular failure male will be soon able to become father to their own genetic children. New drugs such as GnRH antagonists hold promise of leading to a better ovarian stimulation, egg quality and implantation rates. ART advances for infertile couple were among the great medical success of the last century. ART has made huge progress and fast stride towards finding suitable treatment options for each infertile couple. Further consideration for IVF treatment should be re-evaluation of infertility causes, through counseling to continue treatment cycle,[91] ICSI option, antagonist ovulation induction, embryo selection with prolonged cell culture, oocyte donation in old age women, cryopreservation of oocytes are an effective treatment strategy that optimize the final result IVF success. Surrogacy, adoption and oocyte or sperm donor should be considered in advances cases of repeated IVF treatment fail.

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