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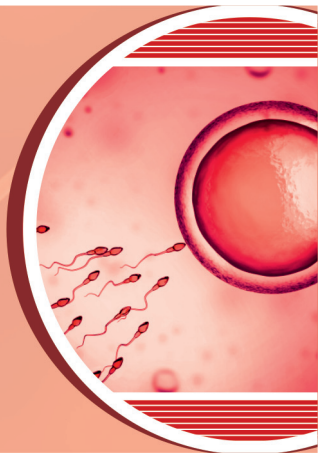
Recent Advances in the Management of Infertility



INTRODUCTION

Infertility is "a disease or dysfunction of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse" (WHO-ICMART glossary). It is a common clinical problem affecting 13% to 15% of couples worldwide although only about a half seek treatment. The WHO estimates the overall prevalence of primary infertility in India to be between 3.9% and 16.8%. The extent of investigations and various modalities of management of infertility have advanced tremendously since IVF was introduced as a treatment for childless couples. In recent years, publicity about infertility treatments has increased, and couples are now more willing to seek advice.

This chapter deals with the magnitude of the problem of infertility in general and the recent advances introduced in the investigation and management of infertile couples with particular reference to Assisted Reproductive Technologies. With this objective this chapter focuses on:

a. Identification of cause(s) and categorization of couples into different groups based on severity of the etiological factor(s) of infertility.
b. Further stratification of patients with regard to expected outcome of management.
c. New ideas and brief overview of ART—current scenario and future thoughts.

Though infertility does not claim an individual's life, it inflicts devastating emotional trauma on the couple—resulting in dissatisfaction, dejection and despair. These couples experience depression, grief, guilt, shame and inadequacy with social isolation.

Infertility Evaluation

Infertility was once considered as just women's problem. But the modern concept of the etiology of infertility connotes equal responsibility to both the partners. It also emphasizes the possible existence of several etiological factors, and that an infertile union may not be the result of a single defect in one partner, but rather the result of manifold factors in one or both partners. The causes of infertility hence are complex and multiple. Identification and treatment of these causative factors are, therefore, essential in the management of infertility. The investigation of the infertile patient must begin with adequate history taking and physical examination of both partners.

The accurate detection of underlying reproductive abnormalities helps in guiding individual management decisions and maximize ART treatment outcomes. Clinical

evaluation of the infertile couple may be grouped into: (a) Clinical evaluation, (b) semen analysis, (c) ovulation monitoring, (d) assessment of tubal patency and tubo-peritoneal defects. Based on the findings of these four basic investigations, all infertile couples can be broadly categorized into three groups:

a. Single defect in one of the partners.
b. Multiple defects in one or both partners.
c. Apparently no defect in either partner (unexplained infertility).

Expectant management is the most appropriate approach for infertile couples with a good prognosis for spontaneous pregnancy, whereas ART provides a valuable treatment option for selected couples with a low probability of natural conception.

Couples with Single Defect in One of the Partners

They can be further categorized into two groups: (i) Defect can be treated easily; such defects in the female partner are ovulatory defect, tough hymen leading to dyspareunia and in the male partner are mild oligospermia or phimosis, (ii) defect is not easily treatable; few such are azoospermia in the male partner or premature ovarian failure (POF) or advanced endometriosis in the female partner.

Couples with single defect in one partner and easily treatable can be treated primarily by a gynecologist and may not be referred to tertiary infertility center for specialized treatment initially. Couples unable to conceive after a reasonable period of treatment may be referred to a specialized center for further investigation and management.

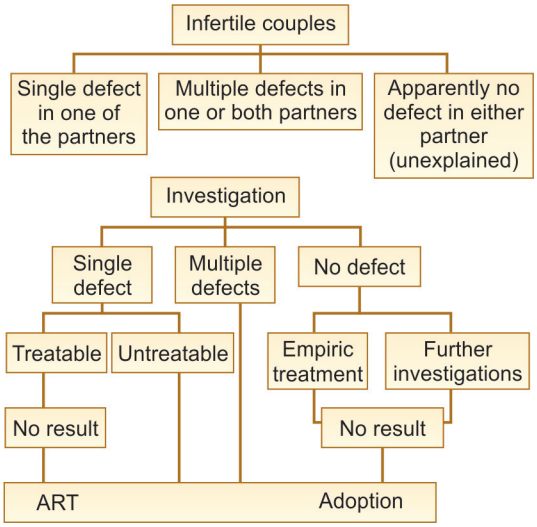
Couples with Multiple Defects in One or both Partners

Couples who present with multiple defects like seminal defect in the husband with tubal block in the female partner or in the same partner (female) tubal block with uterine synechiae should be referred to tertiary center for specialized treatment without any delay.

Couples with Apparently no Defect in Either Partner

Prognosis is better in the women with unexplained infertility compared to other two groups provided: (a) Age of the female partner is less than 35 years, (b) duration of infertility is less than 7 years, (c) menstrual cyclicity is regular.

The proposed stratification and the entire protocol is represented in the following flowcharts:



How to ascertain whether 'easily treatable' or 'difficult to treat'

This can be ascertained by clinical evaluation and a few basic investigations.

Significance of Clinical Evaluation

Clinical evaluation in any branch of medicine consists of four essential parts: (i) Inspection, (ii) interrogation, (iii) examination, (iv) investigation.

Inspection and interrogation with clinical examination offer most of the information necessary to establish the diagnosis. A few basic investigations may help in corroborating the clinical diagnosis. A few examples are detailed below for establishing diagnosis in couples presenting with infertility.

Husband

Inspection: Tall and robust in stature with scanty or absent beard and moustache, sometimes with evidence of gynecomastia indicate diagnosis of Klinefelter syndrome to be confirmed by semen analysis and karyotype.

Interrogation: Male fertility also declines with age, most noticeably after the age of 55, with a concomitant increase in chromosomal anomalies in offspring. The average time to pregnancy if a man is under 25 is just over 4.5 months but nearly two years if a man is over 40 (if the woman is under 25). There is a fivefold increase in time to pregnancy if the male partner is aged over 45 years.

History of mumps orchitis, testicular trauma, undescended testis suggest defect in semen analysis. Sexual dysfunctions like erectile and ejaculatory failure are encountered frequently in clinical practice and their evaluation is mandatory. Diabetes of prolonged duration may be associated with sexual dysfunction. Stress of modern society, changes in lifestyle and food habits may be responsible. Similarly loss of libido and premature ejaculation are other symptoms which require psychological support (Fig. 1.1).

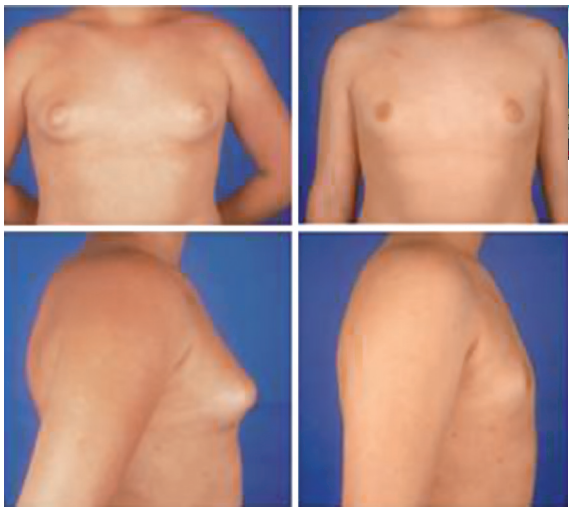


Fig. 1.1: Gross appearance of Klinefelter syndrome—no moustache and beard, tall and robust
Gynecomastia additional feature of Klinefelter syndrome (inset)

Clinical examination: Both systemic and local, to detect any problem that might be the cause of infertility or may modify the management of infertility.

Interrogation: Semen analysis including both morphological and functional tests; if any abnormality is detected, repeat test should be performed after a suitable interval of abstinence.

Wife

Inspection: Obese body habitus especially central with presence of acne and hirsutism may suggest a diagnosis of PCOS.

Interrogation: The most important determinant of a couple's fertility is the woman's age. For women up to age of 25 the cumulative conception rate is 60% at six months and 85% at a year, but conception rates are more than halved by 35 or over. The finite number of oocytes means that age reduces fertility and increases the risk of congenital abnormalities.

Duration of marriage is an important factor because infertility duration more than 7 years may indicate an obstinate infertility factor which may require treatment in the higher order referral units.

Delayed periods (oligomenorrhea), or secondary amenorrhea may indicate PCOS. Secondary amenorrhea with normal secondary sex characters not responding to combination of estrogen progesterone withdrawal treatment indicate uterine synechiae while the similar type of patient responding to withdrawal treatment may be due to PCOS or premature ovarian failure. **Regular menstruating women with cyclicity of 28 ± 2 days rarely have anovulatory or dysovulatory problems.**

Enlisted below are a few common contributory factors of infertility with their salient points that need emphasis during patient–doctor interaction. Primary amenorrhea with normal secondary sex characters not responding to 'withdrawal' is due to Rokitansky-Küster-Hauser syndrome.

Primary amenorrhea with normal or absent secondary sex characters responding to

'withdrawal' may be due to 'mosaic' or 'true' Turner syndrome. History of galactorrhea or anosmia associated with primary amenorrhea, signify hyperprolactinemia and Kallmann's syndrome respectively. Past history indicating tuberculosis in any organ of the body may suggest the possibility of genital tuberculosis.

Progressively increasing premenstrual congestive dysmenorrhea may suggest pelvic endometriosis or adenomyosis.

Deep dyspareunia indicates endometriosis involving in the pouch of Douglas or rectovaginal septum.

Superficial dyspareunia may be due to rigid perineum, tough hymen or frigidity.

Excessive and prolonged bleeding during menstruation indicate uterine leiomyoma while such type of bleeding associated with significant degree of dysmenorrhea may point to a possible diagnosis of adenomyosis.

Clinical examination: Both systemic and local examination should be performed to detect any problem that might be the cause of infertility or that may modify the management of infertility.

Investigation (Fig.1.2)

- Detection and timing of ovulation may be predicted by basal body temperature (BBT), cervical mucus studies, ultrasonography, premenstrual endometrial biopsy and serum progesterone estimation in the mid-luteal phase.
- Assessment of tubal patency by appropriate investigations including hysterosalpingography, sonosalpingography, laparoscopy and/or to find out/rule out specific problems and to assess likely response to therapy.
- Screening for local factors including cervical mucus-related problems and lower genital tract infections, and instituting appropriate therapy.
- Screening for reproductive tract infections including syphilis, Chlamydia, tuberculosis, HBV, HCV and HIV and appropriate management.
- If indicated, appropriate endocrinological investigations are to be performed and appropriate therapy should be formulated.

Few mandatory investigations for the couple include

- Semen profile
- Ovulatory status
- Tubal and tubo-peritoneal defects

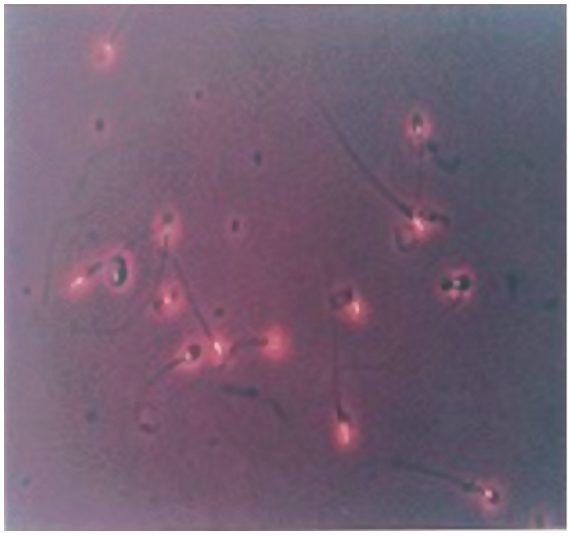
Semen profile

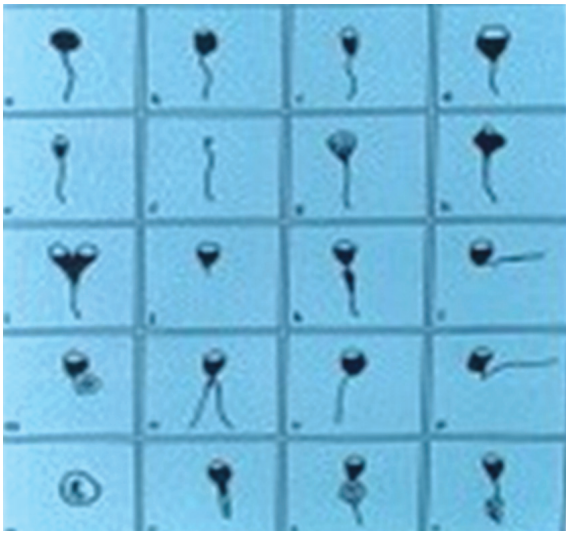
- Defect in liquefaction time—treatable
- Oligospermia—treatable in a few cases
- Oligoasthenospermia—difficult to treat
- OTA syndrome
- Azoospermia.

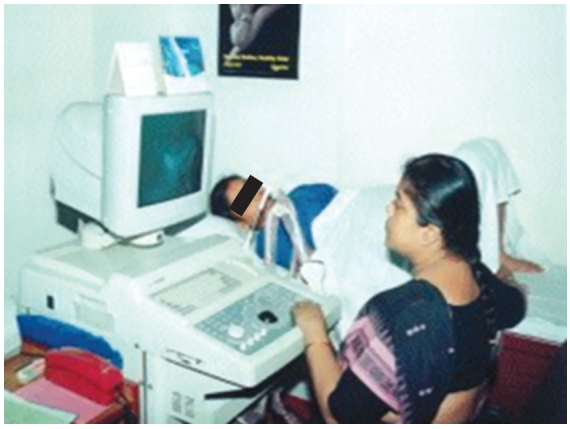
Based on these investigations, all infertile patients may be classified into two sub-groups with regard to their expected treatment outcome: (a) Favorable, (b) unfavorable.

Favorable group indicate those couples who are likely to respond to specific treatment offered to them. Whereas unfavorable group indicate bad prognosis to any form of available infertility management. The classification has been based on: (i) Age, (ii) duration of infertility, (iii) type of defect either in one or both partner; whether treatable or untreatable; whether single or multiple, (iv) ovarian reserve assessed by baseline FSH (D1/2/3, antral follicle count (AFC), anti-müllerian hormone (AMH), (v) the degree of pelvic adhesion—extensive, minimal or no adhesion, (vi) uterine shape, size and cavity—normal or distorted and enlarged (Fig. 1.2). The following table summarizes the parameters for favorable and unfavorable groups of infertile couple.

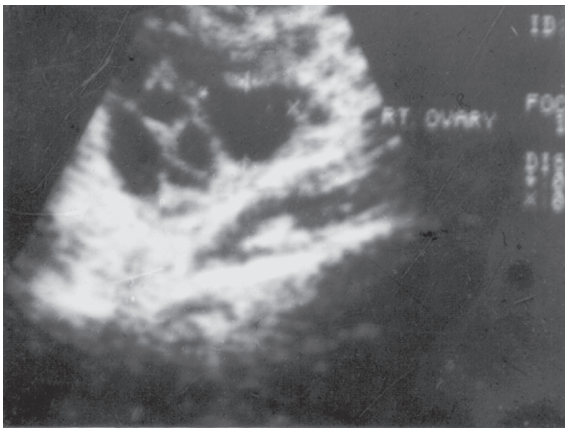
Parameters	*Favorable*	*Unfavorable*
Age	<37 yrs	>37 yrs
Duration of infertility	<7 yrs	>7 yrs
Type of defect	Single treatable	Untreatable or multiple
Baseline FSH	<10 mIU/ml	>10 mIU/ml
BAF	>6	<6
Pelvic adhesions	Minimum or none	Moderate to extensive
Uterine shape, size and cavity	Normal	Distorted and enlarged





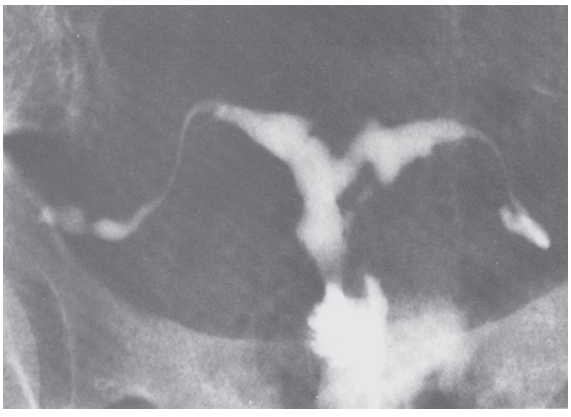


Transvaginal ultrasonography



Folliculometry by TVS

HSG—intravasation of dye into myometriums and lymphatic and terminal hydrosalpinx



HSG—synechiae tubal block

Fig. 1.2: Routine investigations in an infertile couple—semen analysis, detection of ovulation and tubal patency test by HSG

Etiologywise favorable groups of infertile couples consist of

i. Anovulation—commonest is PCOS
ii. Early endometriosis
iii. Unexplained infertility
iv. Mild anatomical defect like tough hymen or phimosis
v. Mild oligospermia but not asthenospermia

Majority of these patients respond to medical or surgical treatment; a few require IUI/ART.

Etiologywise unfavorable groups of infertile couples consist of

i. Advanced endometriosis with or without adenomyosis
ii. Uterine synechiae
iii. Azoospermia/asthenospermia
iv. Dense pelvic adhesions due to any cause. The common causes of dense pelvic adhesions are—endometriosis, tuberculosis, non-specific pelvic infection and post-operative adhesions. Majority of these patients require ART/adoption; a few may respond to medical/surgical therapy.

Management protocol outline of a few commonly encountered infertility problems

The common problems encountered are: (a) PCOS, (b) endometriosis, (c) male infertility, (d) unexplained infertility.

Management protocol of anovulation; commonest condition is PCOS

PCOS women with infertility may present with different clinical presentations and obviously the management varies in each group (*see* Chapter on overview and management of PCOS)

Group A (mild group): These women present with delayed menstrual cycle (32–35 days cycle). They may or may not have family history of diabetes, usually non-hirsute and non-obese. On USG scan the ovaries may be normal in size or enlarged but no thecal hyperplasia. Serum insulin levels may be normal.

These cases are typical clomiphene responders. Adjuncts like bromocriptine, levothyroxin or dexamethasone may be added.

Group B (moderate group): These individuals are mildly androgenised with hirsutism; there may be mild obesity (elevated BMI) and oligomenorrhea (35–45 days cycle). There may be family history of diabetes. On USG scan ovaries are enlarged with increased blood flow; stromal hyperplasia and peripheral cysts (necklace pattern) may be present. Peripheral blood serum analysis may exhibit insulin resistance. These patients may still respond to clomiphene citrate (CC), but better option is to combine CC with insulin sensitizers like metformin or rosiglitazone.

Group C (severe group): This group of PCOS women consist of subjects with typical obesity and stocky build with family history of diabetes or PCOS. Oligomenorrhea (45–60 days) or secondary amenorrhea are commonly associated. Gross clinical evidence of hyperandrogenicity like "HAIR-AN" syndrome (hyperandrogenicity, insulin resistance, acanthosis nigricans) may co-exist. On ultrasound scan, ovaries are typically polycystic with increased vascularity and stromal hyperplasia. Insulin resistance is commonly associated with this group of PCOS women. In addition there may be hyperhomocystinemia.

They are the ideal candidates for: (a) Insulin sensitizing agent (ISA)—metformin with CC or gonadotropin, (b) alternatively they may also be treated with ISA+ovarian drilling, (c) final option should be for downregulation followed by gonadotropin, ISA and IVF. They are highly susceptible for OHSS and care should be taken during ovarian stimulation.

Summary (management of anovulation–PCOS)

- CC with or without adjunct is the first line of management.
- Gonadotropin with or without down-regulation in CC resistant women. This regime is unsuitable for patients who cannot be followed up or monitored during stimulation or treatment.
- Ovarian drilling, an alternative procedure, has some added advantage specially for those patients who cannot be monitored during treatment (gonadotropin stimulation)

- Aromatase inhibitors, though controversial (currently banned in our country), may be considered before expensive gonadotropin or interventional drilling.

For further details of management of PCOS see other related chapters on PCOS.

Endometriosis

Therapies currently available for improvement of fertility

Infertility management of endometriosis involves five different types of options depending on the extent and severity of the disease. These are: (a) Expectant, (b) medical, (c) surgical, (d) combination of medical/surgical, (e) ART.

Expectant management

Expectant management may be possible in early but not in advanced stages, though a few controversies still exist regarding the role of such management. Because, when considered in view of treatment outcome and extension of the disease volume, active rather than expectant management seems to be more convenient. In addition, it has been observed that in early stages also endometriosis has an adverse impact on the fertility potential of a woman. In other words, women in their teens, who are potential candidates for developing endometriosis in future have some inherent defects which are responsible for their subfertility (poor oocyte quality and impaired endometrial receptivity). Figure 1.3 illustrates fertility potential in women likely to develop endometriosis compared to those who are otherwise normal.

Therefore, in early endometriosis fertility improves following some active management like COH-IUI/IVF compared to those in expectant management. Some of the reported figures along with our experience are given below.

	IUI (COH) (n = 22)	*Expectant (n = 25)*
Monthly fecundity	0.15%	0.05%
Cumulative preg. rate	37%	24%

(Feedle et al, 1995)

	No. of cycles	*Clinical preg. n(%)*	*Viable delivery n(%)*
Our series (2002)	35	11 (28.8%)	9 (23.6%)
Heden et al (2000)	223	27%	20.7%

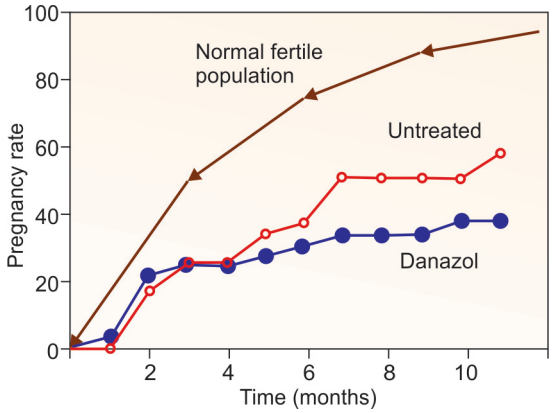


Fig. 1.3: Graph showing fertility potential of women with (treated and untreated) and without endometriosis (stage 1 and stage 2a); Blue—treated endometriosis, red—untreated endometriosis, brown—without endometriosis

Fertility restoration in advanced endometriosis

Five types of treatment options are available, namely:

1. **Medical treatment alone:** Result in terms of fertility restoration is not at all encouraging. This treatment is suitable for relief of pain and to some extent for reduction of disease volume (for details, see chapter on medical management of endometriosis).
2. **Surgical treatment alone:** Reasonable success is expected following this type of treatment. Conservative surgical treatment for restoration of fertility should preferably be performed through laparoscope because:
   i. There is less tissue injury
   ii. Advantage of selecting proper energy source for cauterization or vaporization
   iii. Facilitates aqua-dissection or instrumental rather than finger tip dissection
   iv. Better exposure of pelvis and POD.

   Conservative surgical treatment may be of two types depending on the extent of adhesion and types of lesion (e.g. chocolate cyst, adenomyosis, etc.) existing. Accordingly two types of conservative surgeries are performed in advanced endometriosis,

namely—'function restoring surgery (anatomical restoration of pelvic organs)' and 'debulking surgery (chocolate cyst stripping, lysis of adhesion, adenomyomectomy or myomectomy)'. The first type of surgery is possible when the adhesions are less (filmy) and later type of surgery is indicated when the adhesions are dense with or without additional ovarian or uterine involvement

3. **Surgical combined with medical management, more frequently in combination with GnRH:** In advanced endometriosis a combination of surgical and medical management offers better results than surgery alone. A three step combination treatment of surgery [laparoscopic aspiration of chocolate cyst followed by 3 months long acting GnRH analogue treatment and then again 2nd look laparoscopy (removal of residual endometriotic lesions; sandwich operation)] has been claimed to offer a better pregnancy rate than surgery alone.
4. **ART alone:** Only ART in advanced endometriosis is not a very rewarding treatment.
5. **ART preceded by surgery or medical management:** This combination is expected to offer the best result.

However, in damaged and distorted uterus as in adenomyosis or endometriosis associated with multiple fibroids where endometrial receptivity is expected, to be poor, surrogacy remains the only alternative.

The practical approach to management is summarized in the following paragraphs and algorithm (Fig. 1.4).

Approach to management of advanced endometriosis with infertility primarily starts with assessment of ovarian function. This is because apart from ovarian cortical destruction by the disease process, there also is a compromised ovarian reserve due to prior surgical interventions.

The approach should be as follows:

A. *If the ovarian reserve is not compromised*

and if endometrioma and pelvic adhesions are present, laparoscopic conservative surgical approach is indicated. In the presence of dense adhesions, lysis of adhesions with cyst

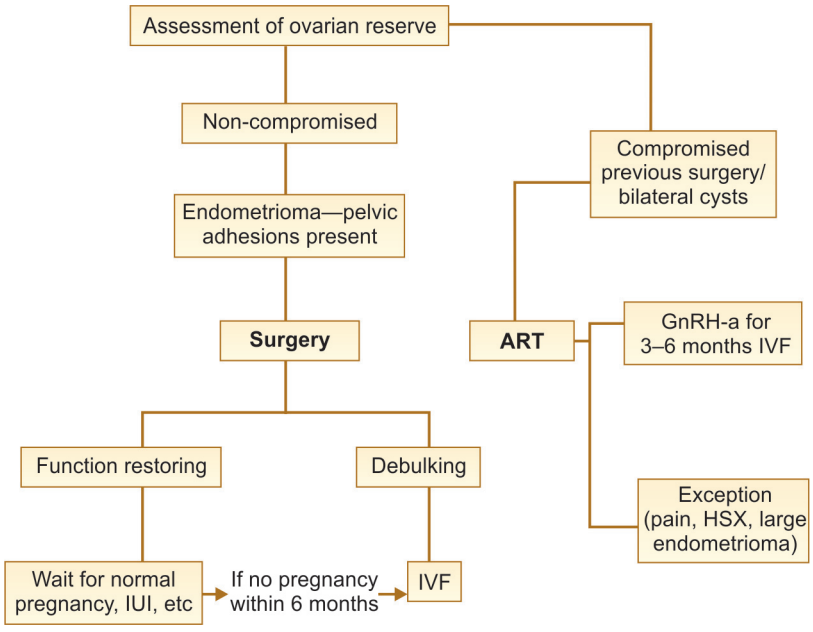


Fig. 1.4: Algorithm of infertility workup in endometriosis

resection are indicated only to make her fit suitable for subsequent IVF. On the other hand, if the adhesions are flimsy and thin—adhesiolysis and restoration of normal pelvic anatomy should be attempted. This is known as 'Function restoring surgery'. If possible, either normal pregnancy or assisted pregnancy through COS (controlled ovarian stimulation) followed by IUI should be advised. If pregnancy is not achieved with these procedures within 6 months, IVF should be advocated. Further delay in starting IVF may result in either recurrence or reappearance of extensive pelvic adhesions.

Conservative surgery is not possible or indicated in women:

i. Above the age of 40 years.
ii. Big adenomyosis (more than 16 weeks gravid uterus size).
iii. Repeated previous surgical intervention.

B. *On the other hand, if the ovarian reserve is compromised*: Either immediate IVF or in case of intractable pain and abnormal bleeding, GnRH analogue (preferably ultra long GnRH-a) followed by IVF is indicated. In case of persistent pain and abnormal bleeding, it is expected that GnRH-a helps in suppressing the severity of the disease and thereafter IVF would be possible. But in practice, sometimes it is very difficult to find a 'disease—remission' period during which IVF protocol can be initiated. In these intractable cases, surrogacy for achieving pregnancy is an alternative procedure. But egg retrieval is also difficult because initiating ovarian stimulation may also become problematic. In such cases donor egg with surrogacy may have to be advised. Unfortunately, for relief of pain and abnormal bleeding, hysterectomy remains the only answer.

Hence in summary, it appears that IVF, preceded by conservative surgery or ultra long GnRH-a therapy, offers acceptable pregnancy rates, in advanced endometriosis with or without associated endometrioma.

Male Infertility

Medical treatment in male infertility consists of hormones (testosterone and hCG) antibiotics and antioxidants. Testosterone and hCG are indicated in oligospermia without astheno or teratozoospermia. In some cases of hypogonadotropic azoospermia (Kallmann's syndrome), a combination of HMG and hCG may be rewarding. But these treatments are expensive and totally unpredictable. Similarly antioxidants, vitamins and antibiotics have been suggested in some cases of asthenospermia associated with infection (leukocytospermia). But the results are very unpredictable and very often unsuccessful. After introduction of PESA/TESE (Fig. 1.5) and ICSI (Fig. 1.6), the importance of medical treatment of male infertility has practically disappeared. But since these methods of assisted reproduction are very expensive, newer research in male infertility is in progress and many pharmaceutical agents (nutraceuticals) are being introduced for trial in the treatment of male infertility. Antibiotics have a positive role in presence of chronic infection of accessory male sex glands.

Sexual dysfunction, in the form of erectile or ejaculatory defect is increasing due to stress and change of social, professional and dietary habits. Medical treatment in the form of sildenafil (viagra), antioxidants and hormones (testosterone) are being used with sporadic success. Semen collection by vibrator, electro

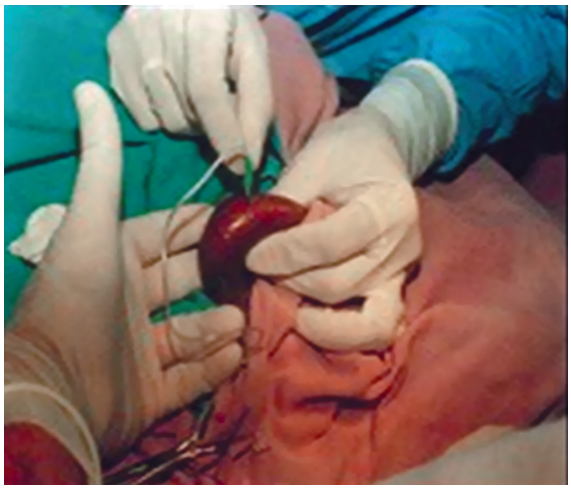


Fig. 1.5: Technique of PESA (per epididymal sperm aspiration) or TESE (testicular sperm extraction)

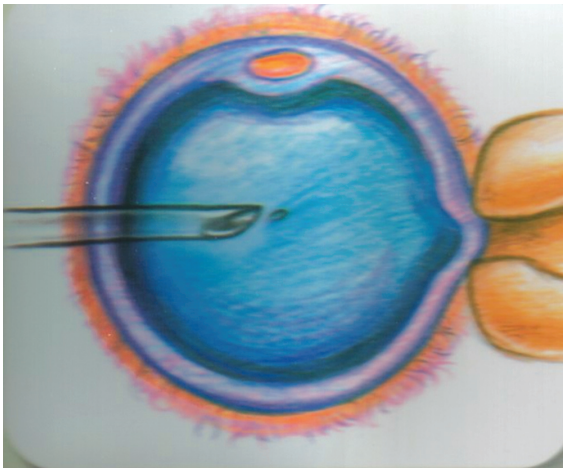


Fig. 1.6: Technique of sperm injection into the ooplasm (injecting needle introduced through zona pellucida into ooplasm at 90° related to position of polar body)

ejaculator followed by IUI, and in extreme cases even PESA/TESE-ICSI may have to be attempted.

The incidence of azoospermia is also increasing. Apart from professional and social stress, changes in dietary habits, environmental influence, late marriage and other factors are contributory for the decline in semen quality all over the world. These men also require either PESA/TESE-ICSI or IMSI or AID. AID is gradually being restricted by law and ethics, hence the number of sperm donors is declining all over the world. This has stimulated research in creating germ or sperm cells through stem cell culture in the laboratory. The research is still in its infancy and has been partly successful in animal models.

Unexplained Infertility

Apart from many other etiological factors one of the most important causes of unexplained infertility is failure of sperm-ovum contact and oocyte fertilization. The possible etiological factors are: (a) Functional defects in spermatozoa, (b) functional defects in the oocyte.

The functional defects of spermatozoa: May exist in: (i) Plasma membrane covering the head, (ii) acrosome deficiency, (iii) nuclear DNA fragmentation. These defects cannot generally be assessed in an average laboratory; therefore their detection is possible in laboratories attached to an IVF unit or in a specialized andrology laboratory. The details of functional defect of spermatozoa have already been discussed in some other chapter of this book. Assessment procedures of different defects are summarized below:

i. ***Hypo-osmotic swelling test (HOST):*** For plasma membrane integrity.

ii. **Test for acrosin competence:** *Acrosin Assay* (biochemical test) and polscopic evaluation of the sperm head.

iii. ***DNA fragmentation test*:** Acridin orange test, *tunel assay* and *comet assay* (fluorescent microscope)

The functional defects in oocyte may be of two types: (a) Defect in zona pellucida, (b) defect in ooplasm.

The site of contact of spermatozoa with oocyte is at the region of zona pellucida protein-3 (ZP-3). If there is an abnormality it can be tested in IVF laboratory by using the 'test' oocyte and proven fertile donor's spermatozoa. After 19 hours of insemination, if there is no pronuclear formation it indicates failure of fertilization.

Ooplasm defect is, however, detected directly by biochemical test and indirectly by failure of development of pronuclear, *nucleolar precursor bodies* (NPB) and meiotic spindle in the ooplasm.

Immunological Defect

There are various types of sperm antigens existing in the seminal plasma as well as on the surface of spermatozoa. Of these, six are sperm specific. The sperm antigens may produce antibodies against: (a) Self—which is known as *autoimmunity*, (b) in cervical mucous, (c) on zona pellucida. The mechanism of production of antibodies on cervical mucous and zona pellucida is known as *isoimmunity.*

In autoimmunity: The sperm antigens may cross the 'blood testes' barrier and pass on to the reticuloendothelial (RE) system and produce antibodies against the self antigens. This self antigen induced self antibodies again cross the blood testes barrier and cause immunological damage to the developing

spermatozoa within the testes. The blood testes barrier allows transfer of antigens and antibodies when the barrier is broken by trauma, infection, injury, etc.

Isoimmunity: Develops after the sperm has been deposited in the vagina following sexual intercourse. The antigens in the sperm surface may produce antibodies locally in the cervical mucous or after meeting the oocyte, on zona pellucida. The antigens will stimulate production of antibodies in the reticuloendothelial system of the body. These antibodies exist in the serum for a long time; but they are not harmful. Only locally produced antibody in the cervical mucous and on the surface of zona pellucida may lead to infertility by affecting spermatozoal movement. This may impair the propelling activity of the spermatozoa and prevent penetration through cumulus covering and zona pellucida of the spermatozoa (Fig. 1.7a and b).

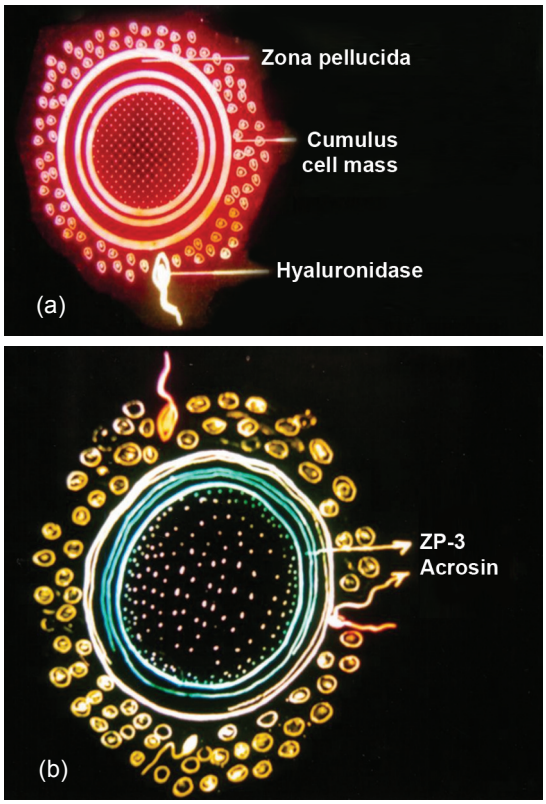


Fig. 1.7: These diagrams illustrate sperm penetration through: (a) Cumulus matrix; (b) Zona pellucida

Management of unexplained infertility

Expectant management, while involving the couple in various types of specialized investigation, it has been observed that a substantial number (20–30%) of patients conceived without any specific treatment.

Active management in unexplained infertility consists of: (a) Super ovulation (ovulation augmentation), (b) IUI, (c) IVF, (d) ICSI.

Ovulation augmentation with ovulation inducing drugs like clomiphene or sometimes addition of one or two ampoules of gonadotropin with CC may enhance chances of pregnancy by production of one or two additional oocytes for fertilization.

The procedure of IUI may help in increasing the chances of pregnancy in three ways: (i) By allowing the sperm-ovum contact as close as possible to the date and time of ovulation, (ii) by bringing the sperm very close to the site at which the oocyte is likely to be fertilized (capacitated sperm in the uterine cavity and fertlizable oocyte in the ampulla part of the fallopian tube), (iii) sperm preparation removes all the antigens on the surface and in seminal plasma and thereby prevents antibody formation either on the sperm surface or on zona pellucida.

IVF offers similar advantages as IUI, with the additional benefit of observing the fertilization process in the laboratory before the embryo is transferred back into the uterus.

ICSI is the safest and best procedure for overcoming immunological incompatibility between sperm and the ovum, addressing the fertilization failure. With this belief some clinics perform ICSI as a routine in unexplained infertility, with possibility of immunological defect in the background.

New ideas and brief overview of ART

Overview is discussed under the following headings:

- Newer developments in the current technology of ART.
- Current scenario.
- Future thoughts and possible breakthrough in ART procedure.

Newer developments, why?

Since 1978, IVF has become a well-established treatment of infertility. Over the years, with continuous effort newer strategies have evolved. But still the current success rate is far below what is expected by the couples and the IVF team.

The gradual evolution of stimulation protocol and success rate in each stimulation protocol over the years from 1978 till 2012 has been represented in the bar diagram (Fig. 1.8).

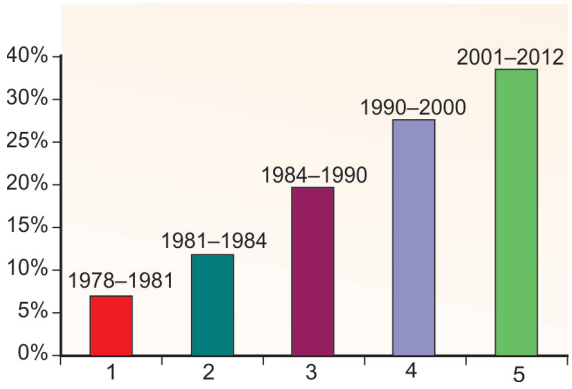


Fig. 1.8: Stagewise IVF success rate with modification of treatment strategies

Current Scenario

Current information about overall success rate in IVF related treatments are as follows:

- 24.7% clinical pregnancies out of all women who undergo IVF treatment (HFEA, 2011).
- 50% of all embryos cultured *in vitro* reach blastocyst stage by D6 (Hardy, et al. 2009).
- ~ 15% of transferred embryos develop into a baby (HFEA, 2000).

Figure 1.9 illustrates current IVF success rate per embryo transfer.

Hence, till recently the trend to improve success rate has been focused on:

- GnRH downregulation with agonist or antagonist—combined with massive amount of gonadotropin stimulation. This protocol is practised because this leads to multiple follicular development yielding more number of eggs—possibility of increased success rate.

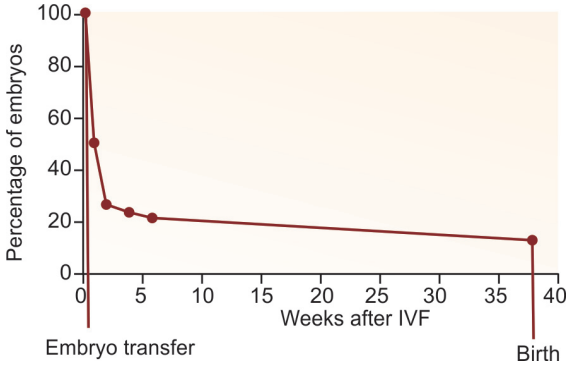


Fig. 1.9: Decreasing survival of human embryos after IVF and embryo transfer. (Data from Dawson et al, 1995; Hardy et al, 2000; HFEA, 2000)

- Multiple embryo transfer (average 3): This is also practised in general by all clinics with the hope of increasing success of implantation.

But the adverse consequences of these procedures are:

- Unacceptable incidence of multiple pregnancies
- Increased cost of treatment
- Complicated treatment procedure

Some of the current figures on the incidence of multiple births following IVF/ICSI are:

- United States 36.5% (2009)
- European data 26.3% (ESHRE report, 2009)
- Our clinic (IRM) 28.2% (2012)

What are the solutions to these problems?

These facts and figures on their own merit have stimulated initiation of new strategies for:

- Improving success rate
- Reducing multiple pregnancies
- Controlling the cost
- Simplification of treatment procedure and optimizing the result with better drug and drug delivery system

And in addition, innovative research is in progress:

- For restoration and preservation of male and female fertility through gamete and gonadal tissue cryopreservation followed by autografting or IVG/IVM of gametes

- Hormonal manipulation and use of adjuncts along with chemotherapy in testicular and breast cancer for gamete preservation

How to improve success rate?

This may be possible by: (a) Improving embryo quality (b) Defining effective endometrial receptivity (c) Optimizing embryo transfer technique. The following diagrams illustrate the current scenario of techniques being employed to improve embryo quality, and the techniques involved in screening of embryos to be transferred back into the mother's uterus (Fig. 1.10).

Some of the important steps of embryo culture and embryo screening are described below:

Great progress has been made over recent years to make pregnancy optimization with single embryo transfer. There are now genetic, morphological and metabolic tools to help us choose the 'best' embryos with the highest chance of developing into healthy babies.

Optimizing Culture Media

a. A promising tool for metabolic assessment of embryo competence lies in identifying the changing nutritional requirement of early cleaving embryo (only pyruvate) to high uptake of both pyruvate and glucose at the blastocyst stage. But this has not yet reached a stage where it can be used routinely.

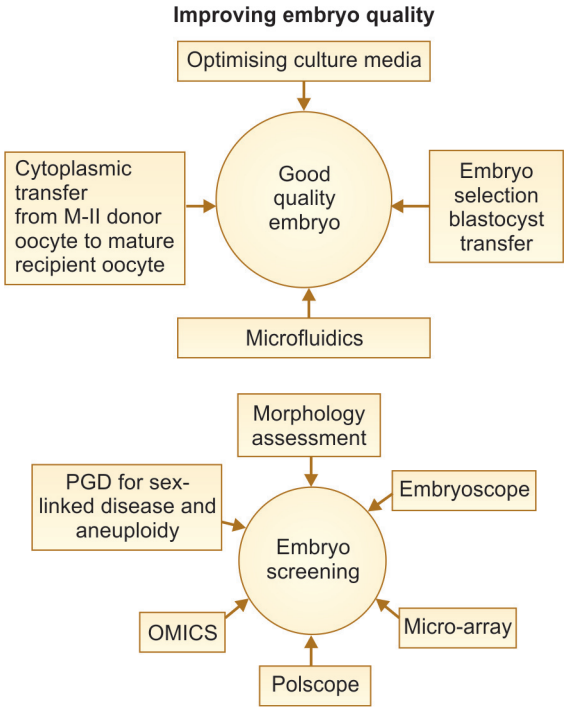


Fig. 1.10: Improving embryo quality

b. Addition of amino acid (Devreker et al, 1998) and supplementation with growth factor (Epanos, et al. 2000) have allowed development of two steps or sequential culture media (Gardner et al, 1998). These new culture media have allowed better development of embryo selection technique.

c. **Microfluidics:** This is a new technology in which the nutritional contents of the media are changed automatically as per requirement of the developing embryo without the need of bringing the embryo out of the incubator. This technology has two specific advantages: (i) Reducing embryo 'stress', because the embryo is not repeatedly brought out of the incubator, (ii) this procedure provides dynamic flow of media which causes gentle agitation of embryo. This may be beneficial for embryo growth and quality. The specific benefits of this procedure of embryo culture are: (i) The technique mimics *in vivo* conditions of fertilization and embryo development, (ii) this provides dynamic flow of media (like *in vivo* intra-tubal fluid) in contrast to static pattern of conventional culture, (iii) it is also like sequential culture media supplemented with varying chemical treatments at prescribed time point, (iv) quality of embryos cultured in microfluidic device are superior to those grown in static system (Heo et al. Hum Reprod, 2010), (v) benefit could also be the result of removal of harmful metabolic products like ammonia, (vi) gentle agitation of embryos may activate intracellular signaling pathways and this physiologically mimics the action of ciliated epithelium.

The procedure is still in the learning stage.

Embryo Screening

a. Blastocyst transfer: Developments in our understanding of embryo biology have

allowed scientists to identify embryos with the best pregnancy potential by observation of progress through the first few days after creation.

Only about 30–40% of embryos continue to develop from day two to day five with considerable attrition rates. While a small percentage of the embryos which do not continue growing may have in fact survived *in vivo* if transferred earlier, many are destined never to implant and thus extended culture provides another means by which to help identify the better embryos from a cohort. It is important however, to appreciate that the process of extended culture does not by itself 'make an embryo better'.

Chromosomally abnormal embryos do not survive up to blastocyst stage (Munne, et al. 1995). However, even in blastocysts, various proportion of abnormal cells have been found which are mosaic (Ruangvutilert, et al. 2000).

Contradictory reports on implantation and pregnancy rates following transfer of blastocysts have been published; 45% and 65% (Milky, et al. 2000) as against 15% and 28% (Huisman, et al. 2000).

b. Embryo screening for sex-linked diseases and aneuploidies (PGD): Genetic assessment with pre-implantation genetic diagnosis.

The genetic make-up of the embryo (and the oocyte in particular) is the major factor influencing implantation and pregnancy rates. Chromosomal factors including aneuploidy (abnormal chromosome number) and translocations, as well as single gene defects, mitochondrial defects and defects of complex inheritance can be important. Even in young women, many embryos are found to have random aneuploidies, while embryos from older women very frequently are found to be aneuploid, and thus unlikely to progress to a normal pregnancy.

Pre-implantation genetic diagnosis with testing of all 23 pairs of chromosomes by a microarray technique (which quantifies the amount of genetic material present of each chromosome) is a relatively new tool, which allows us to differentiate chromosomally normal from abnormal embryos and thus replace only the normal ones on day five, discarding the abnormal embryos. This improves implantation rates. The risk of mosaicism (different cell lines in the biopsied embryo) is 5–7%; so it is important to counsel patients that, to absolutely confirm chromosomal normality, they require an invasive prenatal assessment such as chorionic villus sampling or amniocentesis. Pre-implantation genetic diagnosis can also be utilised for translocations and single gene defects, e.g. BRCA1, BRCA2 and cystic fibrosis.

The techniques used are PCR (polymerase chain reaction) and 'FISH' (fluorescence *in situ* hybridisation). These techniques were first introduced into clinical practice by Handyside, et al. (1990). The technique was used to detect sex-linked diseases, e.g. cystic fibrosis, Tay-Sachs disease. About 200 such sex-linked disorders could be identified by the technique of PCR. 'FISH' technology can detect common fetal aneuploidies (X, Y, 13, 18, 21 and 16).

The biopsy can be taken from: (a) Polar body on d0, (b) living embryo on d3 and (c) blastocyst on d5.

Future thoughts about monogenic disorder from a single cell:

- Current techniques in PGD require investments in resources, staff, finances and time
- More universal techniques are required in PGD which allow many mutations to be simultaneously investigated
- Microarray technology is one of the methods to achieve this objective
- Other methods of amplification consists of whole genome amplification (WGA) or multiple displacement amplification (MDA) (Fig. 1.11).

Upcoming Methods of Oocyte/embryo Screening

The following are the upcoming screening methods:

a. OMICS—e.g. proteomics, metabolomics in spent media
b. Polscope
c. Embryoscope

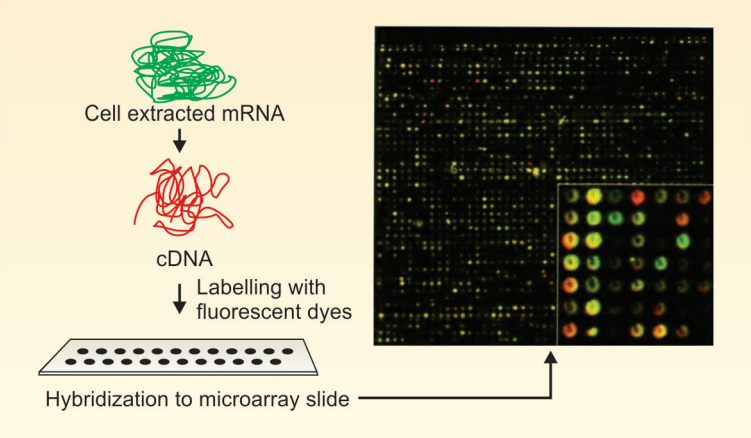


Fig. 1.11: Improving embryo quality

OMICS

The term 'OMICS' indicates methods by which stepwise biological expression of a cell (oocyte or blastomere) at the molecular level can be ascertained. This allows continuous monitoring of viability of oocytes and embryos.

The methodology requires a comprehensive idea of different disciplines in understanding viable embryo genesis including their metabolism, namely transcriptomics, metabolomics, secretomics, etc.

When the embryo is in culture it secretes its metabolites into the spent media. Some of the metabolites indicate viable embryo while some other metabolites indicate non-viable embryo. Identification of the specific metabolite by different techniques, as represented in the following diagram, indicates viability or non-viability of the cleaving embryos. It is entirely a non-invasive technique of embryo assessment. But the disadvantages of the methodology requires establishment of additional laboratory facilities (Fig. 1.12).

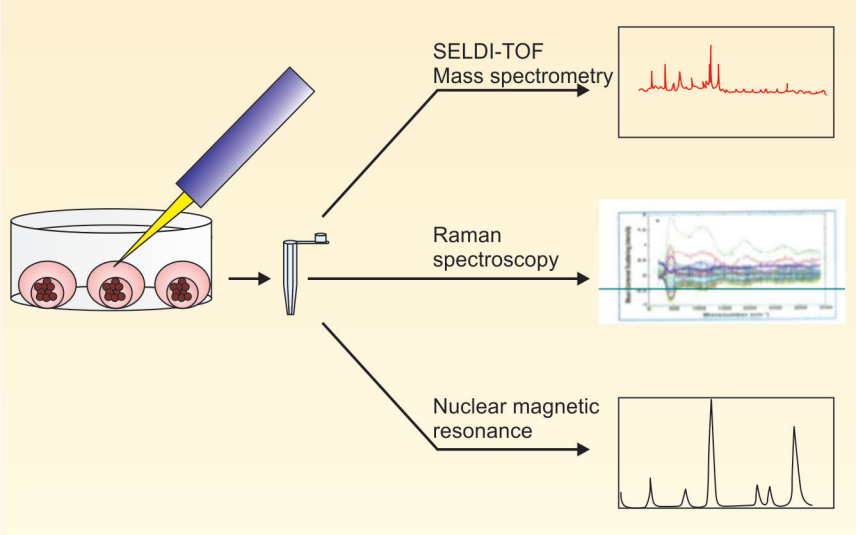


Fig. 1.12: Three techniques to characterise the proteome of single oocytes and embryos, but also to profile the metabolites (metabolome) and proteins secreted by the cells into the surrounding medium (secretome)

Assessment of Oocyte Quality by Polarized Microscope (Polscope)

A rough assessment can be made about the quality of oocyte through polscopic evaluation. The parameters which are assessed are:

a. Visualization of the spindle: Visualization of the spindle is a definite sign of a good quality oocyte. But that does not mean that non-visualization always indicates a poor quality oocyte.

b. Spindle length: Definite measurement of the spindle length (length of meiotic spindle) is related to the onset of cleavage. A length of 14.7 ± 2 micron is associated with anticipated early cleavage, whereas a length of 13.4 ± 2.2 micron suggests probability of late cleavage. A longer spindle length indicates a oocyte of good quality. Shorter spindle length indicates poor quality oocyte.

c. Spindle retardance: Density of spindle illumination indicates spindle retardance. Optimum retardance indicates a good quality oocyte. It is a qualitative test; hence the evaluation depends on observer's experience.

d. Birefringence: This also refers to the quality of the spindle observed under the microscope. The positivity of this test also depends on observer's experience.

e. Polarity: The spindle should lie in the same hemisphere in which the polar body is present (sub-zonal space of the oocyte). If the spindle is located in the opposite hemisphere, the quality of the oocyte is presumed to be unsatisfactory.

Figure 1.13 illustrates the quality of oocyte as assessed from spindle view by a polarized microscope.

Time Lapse Observation by Embryoscope

Embryoscope is a recent microscopic attachment by which embryo development during culture can be observed without bringing the embryo out of the incubator. In other words, this is a non-interventional method of embryo assessment during the entire culture system.

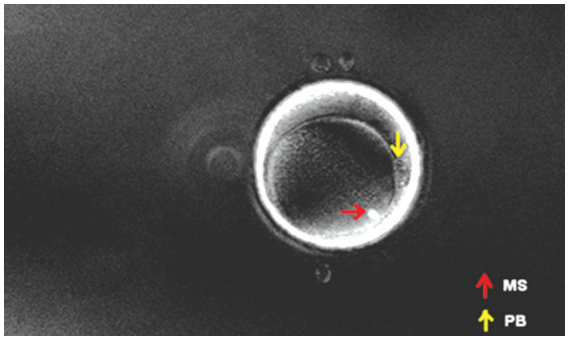


Fig. 1.13: Spindle view

Advantages

The specific advantages are

a. Morphometry (diameter of oocyte and pronuclei) of oocyte and pronuclei can be easily assessed by embryoscope without alteration of culture environment.
b. Oocyte activation can be measured by cytoplasmic waves in post-injected oocytes. Absence of cytoplasmic waves suggests non-activation of the oocyte cytoplasm which may be the cause of failed fertilization following IVF or ICSI.
c. Early cleavage status which is an important criteria of embryo selection can be assessed very precisely by time lapse observation without exposure to outside environment or missing the step by conventional observation.

Hence, this is a revolutionary addition in the research field of ART.

Limitations are

a. Repeated exposure to light when digital images are obtained may have adverse deleterious effect on the cleaving embryos.
b. No significant difference in pregnancy rate has been reported till now following time lapse or conventional intermittent observation.
c. It is a costly affair without marked increase in success rate following ART.

Reducing multiple births and controlling the cost: These objectives can be achieved by: (a) Single embryo transfer, (b) mild approaches of ovarian stimulation; modified natural cycle (MNC) protocol.

Single embryo transfer (SET)

The requirements of single embryo transfer are:

a. The woman must be below the age of 35 years (Gerris et al, 1999; De Surter et al, 2002).
b. Assurance about culture conditions.
c. The embryo quality should be optimum.

This procedure allows cost-control with availability of 'top' order spare embryos for freezing and avoids the perinatal and maternal morbidity inherent to multiple births.

Other short protocol/with antagonist mild approaches of ovarian stimulation

The indication of this type of protocol is specifically suitable for young patients who are potential hyper-responders to conventional COH (PCOS).

Briefly, the protocol consists of

- FSH/HMG from D2
- USG scan from D6/D7
- Adjust the dose of gonadotropin/add antagonist
- hCG trigger

Results

In our experience results are similar to conventional long protocol downregulation but with substantial cost reduction.

Simplification of Current Treatment Protocol

The greatest disadvantage of the current treatment protocol using recombinant FSH or uHMG is that it is a very extended treatment regime. The patients are often distressed by daily injections. Therefore, there are many dropouts. The 'dropout' rates have been estimated to be around 50% even when the treatment costs were reimbursed (Land, et al 1997; Shroder, 2009).

Therefore, a 'patient centered approach' is being attempted. The objectives of this approach are: (a) Shorter treatment cycles, (b) shorter duration of stimulation, (c) less number of injections, (d) less complex medications, no reconstitution, no mixture. For these a trial has been given with a drug which has been designated as "Corifollitropin".

"Corifollitropin alpha" is a recombinant fusion molecule of FSH and the carboxy-terminal peptide (CTP) of hCG-β subunit. The drug is also known as rFSH-CTP (Fig. 1.14).

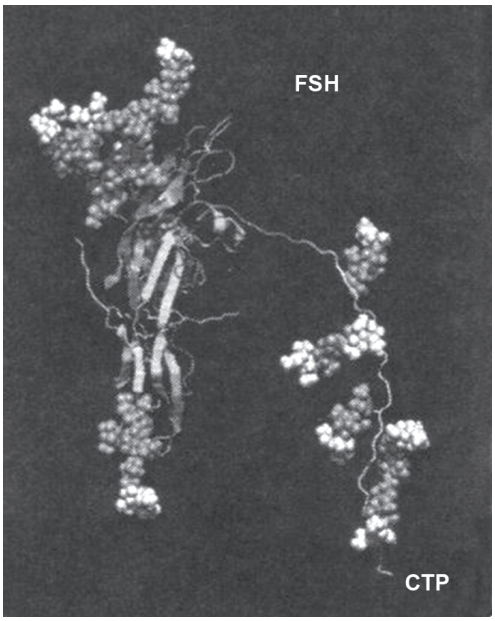


Fig. 1.14: Corifollitropin alpha—FSH-CTP

The specific advantages of rFSH-CTP is that it can initiate and sustain multifollicular growth for 1 week. In other words, single SC injection of FSH-CTP on D2 can replace the first 7 daily injections of rFSH.

Defining Endometrial Receptivity

In a normal menstrual cycle, endometrium remains receptive for blastocyst implantation for a short period only—i.e. from D18 to D23 in unstimulated and D16 to D20 in a stimulated cycle. Receptivity is generated through interaction of various biochemical and physiological molecules in different endometrial compartments. Receptivity varies from woman to woman and in the same woman from cycle to cycle. Hence it is very difficult to identify 'markers' of receptivity in the conception cycle by non-interventional methods. The commonly quoted markers are:

- Well developed pinopodes—filamentous projections from surface epithelium of the endometrium.

- Expression of certain biochemical ingredients from the endometrial surface, e.g. integrin, cadherin, selectin, etc.
- Immunomodulatory changes within the endometrium—infiltration of pro-adhesive cytokines and growth factors replacing antiadhesive cytokines and NK-cells. The commonly observed proadhesive cytokines are IL-3, 4, 5, 6, 10, 13, whereas the anti-adhesive cytokines are IL-12, 18.

Some of the markers demonstrated by electron microscope and immunohistochemistry are shown in Fig. 1.15.

Gamete donation and surrogacy have become more acceptable over the past decade.

Future thoughts and possible breakthrough

There are two possible breakthroughs in ART procedures in near future:

a. Restoration and preservation of male and female fertility through gamete and gonadal tissue cryopreservation followed by auto-grafting or IVG/IVM of gametes.
b. Therapeutic application of outcome of stem cell culture.

Restoration and preservation of male and female fertility potential in situations like:

- **Cancer chemotherapy or radiotherapy:** Cancer is not uncommon in young people, with approximately 1 in 570 young girls and women being diagnosed with cancer before the age of 35 years. For many young cancer patients, treatment offers a very real chance of cure. Also being able to have children in the future is a genuine and appropriate expectation. Many cancer treatments, especially chemotherapy and pelvic radiation, can cause ovarian failure. Such ovarian failure is often temporary with spontaneous resumption of menses, but it can be permanent, especially in women in the later reproductive years. Even when menstruation and ovarian function return after chemotherapy is completed, there is still a very real risk of subsequent early onset of ovarian failure.

Various options are available to young women for fertility preservation before commencement of chemotherapy or radiotherapy which include oocyte freezing, embryo freezing and ovarian tissue cryopreservation.

Ovarian tissue cryopreservation is an option to preserve reproductive potential in patients who must urgently undergo

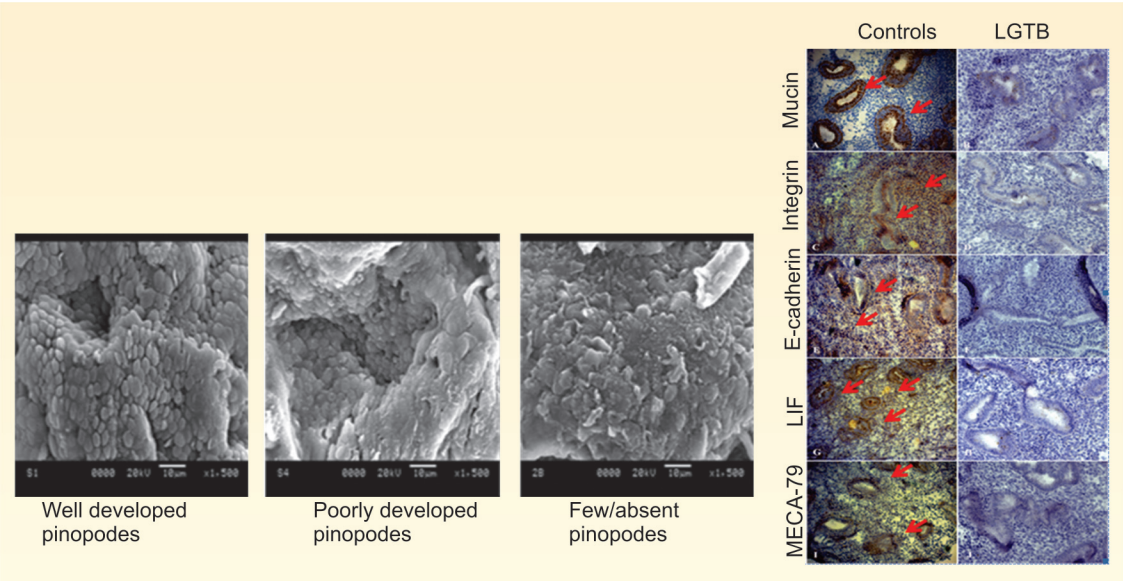


Fig. 1.15: Markers of endometrial receptivity

aggressive chemotherapy and/or radiotherapy or who have other medical conditions requiring treatment that may threaten ovarian function and subsequent fertility. Ovarian tissue cryopreservation may be the only option available to prepubertal girls undergoing such treatments.

Ovarian tissue freezing provides a potentially huge supply of oocytes in the cortical strips.

Till 2014, a total of 26 healthy children have been born as a result of cryo-preserved ovarian tissue. Despite the wider application of ovarian tissue storage for fertility preservation, the relatively small number of babies born suggests that far fewer women after their cancer treatment are probably taking up their options for pregnancy. Nevertheless, the babies born worldwide so far indicate that it is a viable option for fertility preservation.

And in addition

- Oocyte cryopreservation for future use is indicated for:
  i. Oocyte donation
  ii. Cost reduction
  iii. Prevention of OHSS.

Methods of preservation of male fertility potential following chemotherapy

This is being attempted through: (a) Cryopreservation of gametes and gonads, (b) hormonal manipulation and (c) cryoprotectant adjuvants.

Cryopreservation of male gametes and gonads

This is possible through cryopreservation of testicular biopsy material followed by autografting and *in vitro* maturation of human spermatozoa after the disease is cured. Sperm freezing should always be considered for adolescent males and young men about to commence chemotherapy or testicular surgery. If producing a sample is difficult, then testicular biopsy under local or light general anesthesia can be considered. Currently much research is going on to find ways to mature sperm *in vitro* from very immature forms.

After recovery, autologous germ cell transplantation back to adult testes hopefully results in initiation of spermatogenesis. (Hovatta, 2001). This idea has been practically possible in animals. Testicular cells, including germ cells, have been injected *in vivo* and the transplanted germ cells were present 4 weeks after transfer (Schlatt et al, 1999).

Hormonal manipulation and cryoprotectant adjuvant therapy

It has been observed that chemotherapeutic agents cause more gonadal damage to post-pubertal men than in pre-pubertal boys. Using this principle, GnRH agonist or testosterone can be used to suppress active spermatogenesis during chemotherapy. Resumption of spermatogenesis has been observed following cessation of chemotherapy.

In addition, cryoprotectant adjuvants like lipoic acid, lycopene, YMG (herbal drug) used along with chemotherapeutic agents show improvement of sperm parameters in animal model.

Preservation and restoration of female fertility

Embryo cryopreservation is possible but oocyte cryopreservation is relatively difficult. Oocyte cryopreservation is indicated in young cancer patients undergoing chemotherapy or radiotherapy to retain their fertility potential after recovery. But it is difficult to cryopreserve mature oocytes. Less than 40% oocytes survive thawing and only 20% surviving oocytes mature to Metaphase-II. (Toth et al, 1994).

The success rates in oocyte cryopreservation have been cited as low and are debatable, with literature review of 21 studies in peer-reviewed journals revealing a mean survival rate of 47%, fertilization rate of 52.5% and mean pregnancy rate/thawed oocyte being 1.52–1.8%.

Therefore, cryopreservation of immature oocyte at earlier stages of development followed by *in vitro* maturation (IVM) has been investigated. But the results are not very satisfactory. However, a three-stage system of approach for IVM is being planned and investigated. This three-stage system consists of—(a) Initiation of *in vitro* growth of primary and primordial follicles in pieces of ovarian

cortical tissue (Hovatta et al, 1999), (b) further *in vitro* growth (IVG) of enzymatically or mechanically isolated preantral follicles, (c) retrieval of immature oocytes from small antral follicles and then *in vitro* maturation (IVM) (Fig. 1.16).

But there are problems. Problems are: (a) Human ovarian stromal tissue is dense. Hence, mechanical isolation of follicles is time consuming, whereas enzymatic isolation may be damaging for the follicles (Abir et al, 1999), (b) human follicles have a long maturation period (about 6 months *in vivo*) and are large when fully grown compared with mouse follicles (20 mm vs 0.6 mm), (c) out of 2 million follicles at birth, only 400 are likely to ovulate. Rest undergo atresia, (d) the development of culture system to support physical and nutritional growth of human follicles is a challenge.

Societal Fertility Preservation

Many women in their late reproductive years without a partner may wish to preserve oocytes for the future. As survival of oocytes after freeze-thawing improves, this becomes more of an option, but it must be remembered that there is a high rate of attrition from oocyte to usable embryo.

Therapeutic Application of Outcome of Stem Cell Culture

One of the significant objectives is to create male and female gametes through stem cell culture. This may avoid the necessity for gamete donation in case of azoospermic husbands or for wives with premature ovarian failure.

This is being experimented in animal models and some success has already been achieved. The ongoing experiments are:

a. From embryonic stem cells through embryoid bodies (Geijessen et al, Nature 427; 148–54 (2004)).
b. From spermatogonial stem cells (adult stem cells) (Hiroshi Kubuta, Proc National Acad of science, 2001).
c. From ovarian stem cell (adult stem cell) (Bokovsky et al, Rep. Biol Endocrin, 2005).

The procedure involves continuous growth of embryonic stem cell in specialized suspension culture. These cells grow continuously. During the growth it has been observed that there is an aggregation of some cells with genetic signature of primitive germ cells (PGC). These cells are precursors of either oocyte or spermatocyte. When these cells are further grown, they become either adult spermatozoa or adult oocyte. This experiment has been carried out in animals. Their potential of fertilizability *in vitro* has not been observed as yet. Figure 1.17 illustrates the growth of germ cell from embryonic stem cells.

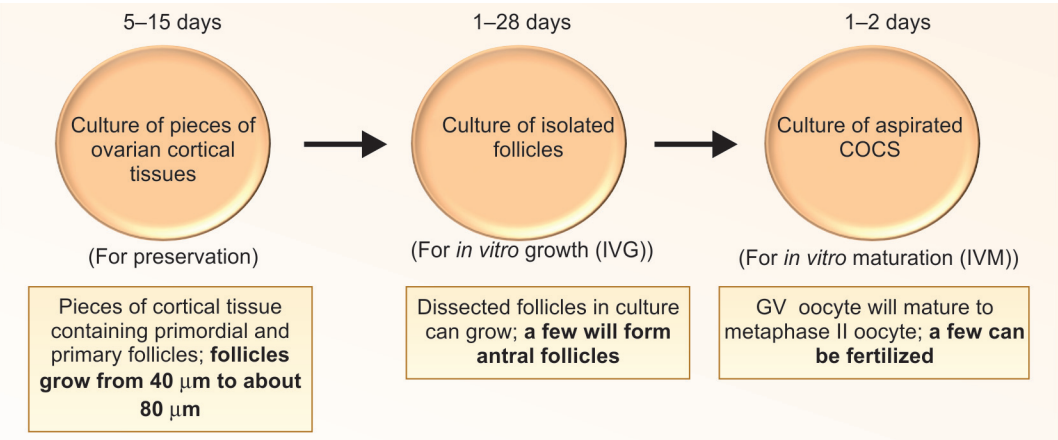


Fig. 1.16: Cryopreservation of immature oocytes in ovarian cortical tissues followed by *in vitro* growth (IVG) and *in vitro* maturation (IVM)–animal model

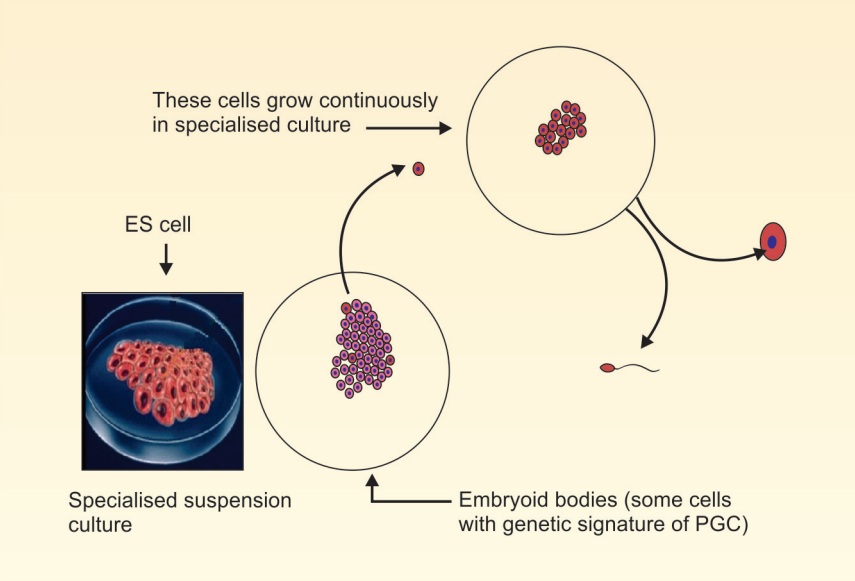


Fig. 1.17: Germ cell generation (oocyte and sperm) from stem cell culture *in vitro*

Immediate benefits of creating human eggs and sperms from stem cell are

a. This avoids use of donor sperm or egg in assisted reproduction
b. Helps preservation of fertility in men and women awaiting chemotherapy
c. Currently preservation of gametes is being done by freezing sperm cells, testicular or ovarian cortical tissues, followed by IVG and IVM
d. Freezing sperm stem cells or ovarian stem cells is better than freezing sperm or testicular and ovarian tissues. This is because sperm 'stem cells' which are 'diploid' can stand cryopreservation better than testicular sperm cells which are 'haploid'.
e. Sperm stem cells may enhance survival of endangered species of animals of valuable livestock.

Conclusion

Over the past few years there have been numerous scientific developments that have advanced our understanding of infertility and provided us with the tools to improve both the success of infertility treatments and their safety. Greater insight into oocyte and embryo biology may drive further improvements in fertility treatments and perhaps, ultimately, the prevention of infertility.

Even with the current knowledge many unfulfilled objectives of IVF are still unknown and research is still continuing to upgrade our knowledge to achieve the objectives which are still not very clear in the field of reproductive medicine.

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