CHAPTER 60

Assisted reproduction

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Introduction

Prior to 1960, it appears that fewer than 20% of couples who presented with infertility subsequently conceived; in fact, those conceptions which did occur were considered to be mostly unrelated to treatment [1]. Subsequently, the prognosis improved with the introduction of effective methods for ovulation induction. This was associated with inspired discoveries and developments in understanding the hypothalamo-pituitary–ovarian axis leading to the current improved level of knowledge regarding events concerning folliculogenesis, oocyte release and luteal function in the ovarian cycle. Further significant advances during the 1960s and 1970s included: the introduction of laparoscopy as a primary investigative tool; the development of sensitive, specific and rapid hormone assays; the appreciation of the role of non-gonococcal anaerobic organisms such as *Bacteroides fragilis*, and later others such as *Chlamydia trachomatis*, in the causation of pelvic inflammatory disease; the recognition of hyperprolactinaemia and its association with infertility; the establishment of donor semen banks; the development of microsurgery (initially on the female and subsequently on the male genital tract), hysteroscopy and endoscopic procedures; and the detection of antibodies against gametes. Such advances have improved considerably the chance of patients achieving a pregnancy, and the subsequent understanding of human infertility has enabled the rational introduction of techniques to assist human reproduction.

The integration of assisted reproductive methods with the aforementioned developments in the comprehensive management of infertility has improved the potential prognosis to beyond 75% of couples who can now be successfully treated to achieve at least one live birth. In fact, the main limiting factors to the successful treatment of infertility are no longer technical but relate to the age of the female partner [2], expense, ethical considerations and certain social aspects. These latter concerns have led to certain public anxieties in many countries and a perceived need to introduce legislative constraints in both service and research aspects of assisted reproduction.

Modern treatments to assist conception may be regarded as treating specific problems (e.g. reconstructive pelvic and tubal microsurgery), general problems, which may be multifactorial or poorly understood (e.g. ovarian stimulation in women who appear to be ovulatory) or substitute (i.e. where gametes or embryos are donated). Ideally, these modes are best carried out in a single unit structured to provide a comprehensive approach to infertility management (Table 60.1). A simple assessment of the effectiveness of treatment modalities can often be difficult due to varying selection criteria of patients used by different authors, and a definite background pregnancy rate observed in the absence of treatment [3]. The systematic evaluation of treatments in controlled clinical trials is therefore vital.

Ovarian stimulation and ovulation induction

The use of drugs to either stimulate ovaries in women already ovulating spontaneously, or to induce ovulation that otherwise would not occur, has become the cornerstone of many treatments in the field of assisted reproduction. At the one extreme, the strategy to promote ovulation in women wishing to conceive by intercourse often involves the development of only a single Graafian follicle to minimise the risk of a multiple pregnancy. At the other extreme are strategies used in *in vitro* fertilisation (IVF) programmes which are designed to result in the development of several follicles to maximise the yield of oocytes, with the risk of multiple pregnancy being minimised by restricting the number of embryos returned to the uterus. The following

Table 60.1 Specific facilities required for the comprehensive management of infertility, preferably located within a single unit functioning every day.

Consultation	Both partners
Counselling	Information Emotional support
Coordination	Senior nurse Tests/instructions/results
Laboratories	Andrology Embryology Cryopreservation Hormone assays Ultrasonography and radiology
Results	Group meeting each afternoon Computer and hard-copy data registers Regular data analysis and evaluation
Treatment	Areas and facilities
Semen	Collection rooms
Theatre	Oocyte recovery/transfers Endoscopy facilities Ultrasound facility Operating microscope

section summarises the approaches, and particularly the drugs, used in improving or achieving ovulation in a range of situations.

Bromocriptine (see also Chapter 10)

Hyperprolactinaemia is an important cause of anovulatory infertility, occurring in 15–20% of patients with amenorrhoea. Up to one-third of these women may have a prolactin-secreting pituitary tumour [4] which should be identified by computed tomography (CT) scan or magnetic resonance imaging (MRI).

Treatment of infertility associated with hyperprolactinaemia is usually done by the administration of the dopamine agonist, bromocriptine, which acts by decreasing the production and secretion of prolactin by the lactotrophs. Bromocriptine causes a reduction in circulating prolactin concentrations within a few hours that can last up to 12 hours [5], and so the drug is usually given twice a day. However, recent evidence suggests that once-daily administration may be effective in many patients.

The administration of bromocriptine is usually very effective when given to carefully selected hyperprolactinaemic women with amenorrhoea, conception occurring in over 80% of cases [6,7]. The taking of bromocriptine by ovulatory infertile women reduces the serum prolactin concentrations to that below the mean for the normal population and pregnancies do ensue [8], but the empirical use of bromocriptine in the absence of clear hyperprolactinaemia is not common and is not generally recommended. Newer dopamine agonists such as quinagolide and cabergoline may be better tolerated [113], but experience of their safety for conception is more limited.

Clomiphene citrate

Clomiphene citrate is an anti-oestrogen which has been used widely in the induction of ovulation for many years, having been first introduced clinically in the early 1960s. It has no progestational, androgenic or antiandrogenic effects. However, it is not without its problems, having an apparent adverse effect upon cervical mucus quality and endometrial morphology [9] and being associated with an increased rate of pregnancy loss [10,11].

Clomiphene is usually given from the early part of the follicular phase, for example from day 2 or 5 of the menstrual cycle, for 5 days, and is thought to work predominantly by eliciting an increased secretion of endogenous follicle-stimulating hormone (FSH). Accordingly, clomiphene is less effective in patients with anovulation associated with suppressed gonadotrophin levels typical of hypothalamo-pituitary insufficiency. Interestingly, the response to clomiphene, in terms of the secretion of FSH, can be used as a bioassay of the ovarian reserve of women [12] and as a predictive marker of their ability to respond to ovarian stimulation [13].

The administration of clomiphene can be used successfully as first-line treatment for women with anovulation associated with polycystic ovary syndrome [14], with those resistant women then often gaining benefit from the use of human menopausal gonadotrophin (hMG). Oligomenorrhoeic women can often do well with treatment by clomiphene, with certain workers advocating its use as an initial therapy in cases of unexplained infertility [15]. Clomiphene can also help correct cases of luteal phase dysfunction and inadequate progesterone secretion by inducing additional luteinising hormone (LH) receptors in the corpora lutea during the mid-luteal phase [16].

There appears to be no known teratogenic effects of clomiphene given to induce ovulation, and a multiple pregnancy rate of approximately 5% is commonly reported.

Menopausal gonadotrophins

Since the initial full report on the use of FSH extracted from human menopausal urine for the treatment of anovulatory infertility [17], numerous reports have appeared in the literature showing the benefits and limitations of the drug.

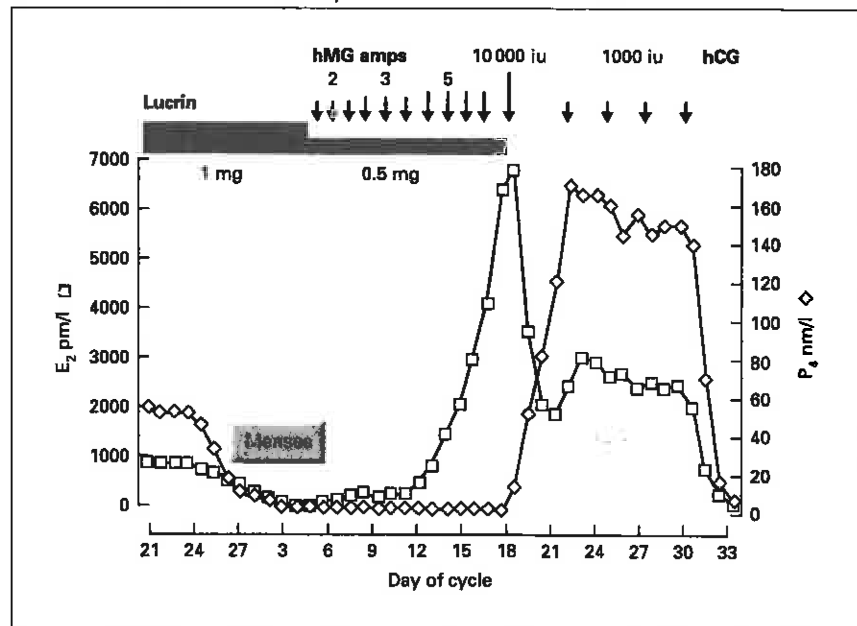

Fig. 60.1 Ovarian stimulation for *in vitro* fertilization (IVF) using leuprolide acetate (Lucrin) for pituitary desensitisation prior to human menopause gonadotrophin (hMG) stimulation. E_2 (□—□); P_4 (◇—◇).

The commercial preparations currently available contain FSH and LH in a 1 : 1 ratio, although there may be human chorionic gonadotrophin (hCG) present [18] and significant variation in the amount of bioactive FSH in different batches [19]. A major difficulty in the use of human menopausal gonadotrophin outside of an IVF programme is the controlled stimulation of a single follicle to minimise the risk of ovarian hyperstimulation and multiple pregnancy. The gonadotrophins do not exhibit a simple dose response, but are said to require a minimum 'effective daily dose' below which there is no measurable effect [20], and there is wide variation in the response of individual patients. Older reports highlight the difficulty early workers had in monitoring the response to menopausal gonadotrophins without the availability of a rapid oestrogen assay or real-time ultrasound, lamenting the fact that clinicians had to 'rely upon somewhat less reliable parameters such as ferning of cervical mucus, vaginal cytology and daily pelvic examinations' [21]. Not surprisingly, there was a significant level of hyperstimulation seen and multiple pregnancies did occur. The current availability of sophisticated monitoring facilities such as rapid oestradiol assays (often providing results within 2 hours) and real-time ovarian ultrasonography has proved of great benefit in monitoring the response of women to hMG therapy, but has not totally eliminated the occurrence of multiple pregnancies, leading to the development of procedures such as selective termination [22] with all its ethical and philosophical problems. One particularly difficult group of patients to manage are those with polycystic ovaries, usually presenting after unsuccessful attempted treatment with clomiphene citrate. These women often exhibit a higher incidence of complications associated with an exaggerated ovarian response following treatment with menopausal gonadotrophins [23]. Strategies using a low-dose regimen, commencing on 1 ampoule per day and only increasing by 1/2 an ampoule if required, appear to be effective in obtaining uniovulatory cycles and can give rise to cumulative pregnancy rates of up to 60% over 6 months [24]. However, excessive concentrations of basal LH in some of these patients can cause an increased incidence of pregnancy loss.

Anovulatory women with no endogenous follicular activity appear to do better with treatment by menopausal gonadotrophins than do those women with follicular activity but disordered ovulation [25,26]. However, the treatment of ovulatory women by menopausal gonadotrophins coupled with other procedures, such as artificial insemination [27], can yield good pregnancy rates, and may be regarded as first-line treatment before progressing to IVF or gamete intra-Fallopian transfer (GIFT).

Gonadotrophin-releasing hormone agonists

The pulsatile intravenous administration of gonadotrophin-releasing hormone (GnRH) agonists is an effective means of treating anovulation by eliciting endogenous gonadotrophin release. The use of automated pumps makes the administration of GnRH agonists much easier to manage, and the treatment has a high success rate and a low incidence of multiple pregnancy [28].

Within an IVF programme, the GnRH agonists can be used either to elicit a flare of gonadotrophins to augment

the administration of exogenous gonadotrophins [29], or achieve pituitary desensitisation to prevent an endogenous LH surge [30]. The flare regimen involves commencing both the analogue and gonadotrophin together at the beginning of the cycle when the analogue will initiate pituitary release of gonadotrophins. A typical desensitisation protocol is shown in Fig. 60.1 and involves commencing Lucrin (Abbott Laboratories) 1 mg s.c. daily in the mid-luteal phase of the preceding cycle. Pituitary desensitisation is usually achieved by day 3–5 of the ensuing cycle and is demonstrated by serum concentrations of LH <5 iu/l and E_2 <200 pmol/l. Thereafter, 0.5 mg Lucrin daily will maintain suppression and hMG injections are given daily. Spontaneous LH surges do not occur with this regimen, which enables the follicles to grow to their full size by delaying the ovulatory trigger [31] until a cohort of follicles have reached 20 mm or greater.

Pituitary gonadotrophin

An early source of FSH was human pituitary glands from cadavers, utilising a range of methods to extract the gonadotrophin from the tissue. The FSH proved valuable initially with pregnancies being established in anovulatory women [32,33]. However, the subsequently transmission of Creutzfeldt–Jakob disease in patients using growth hormone preparations from the same source resulted in the suspension of clinical programmes around the world for fear of infection [34], and the occurrence of deaths following the use of the pituitary gonadotrophin have since been reported [35].

Purified and recombinant human FSH

The removal of unwanted LH from human menopausal gonadotrophin has resulted in a purified form of FSH (Metrodin; Serono Laboratories) which has been used to induce ovulation successfully in cases of polycystic ovarian disease where there is an abundance of endogenous LH relative to FSH [36] and for stimulating follicular development in normal ovulating women [37]. Furthermore, this purified FSH has been used successfully to induce multifollicular development in unselected IVF patients even when endogenous LH secretion is reduced by the administration of a GnRH agonist [38,39]. The use of a highly purified urinary FSH preparation (Metrodin HP; Serono Laboratories), which is practically devoid of LH activity [40], in cycles following pituitary desensitisation has also given good results. This has led to the conclusion that stimulation of the ovaries with exogenous FSH alone is effective, that only very low levels of endogenous LH are required in conjunction with FSH for the stimulation of follicular development and ovarian steroidogenesis, and that supplementation with exogenous LH is not needed. Studies using pituitary-desensitised rhesus monkeys have also shown that additional LH is not required for folliculogenesis and that the presence of LH with FSH in a 1 : 1 ratio during the preovulatory interval, as occurs with the administration of menopausal gonadotrophins, may even impair gametogenic events in the periovulatory period [41].

Recombinant human FSH is now available as a commercial preparation, being produced *in vitro* by genetically engineered Chinese hamster ovary cells in which the genes coding for the α- and β-subunits have been inserted. This highly purified form of FSH has very similar pharmacokinetic features to urinary FSH [42] and pregnancy rates within an IVF programme are comparable [43]. The use of recombinant FSH is likely to supersede that of any of the urinary preparations in time, given its purity and ease of production, although its relative cost will be a debatable point in many parts of the world.

Growth hormone

There is now much evidence to show that growth hormone can directly or indirectly modulate ovarian steroidogenesis. The availability of human biosynthetic growth hormone has therefore made its use as supplement feasible. The most impressive results following cotreatment with growth hormone in maximising the ovarian response and minimising the amount of gonadotrophin required to achieve that response, appear to be with amenorrhoeic patients [44,45]. However, indiscriminate administration to IVF patients showing a poor response to gonadotrophins is not useful [46], echoing the message that careful patient selection is crucial.

Artificial insemination

Male partner's semen

Currently there is a renewed interest in AIH (artificial insemination by husband) for all cases of infertility not due to tubal disease. Although the intrauterine insemination of whole semen has been reported in the past [47], the modern process of AIH is based on the intrauterine insemination of 'washed' spermatozoa [48] arising from the technical developments and improved understanding of reproductive processes involved with IVF-related procedures. The procedure has already developed an established place for the management of infertility due to cervical mucus problems (antisperm antibodies impairing sperm penetration, absence of mucus, undefined hostility and cases displaying poor sperm–mucus interaction), oligozoospermia where this is not complicated by asthenospermia, and retrograde ejaculation

and psychosexual disorders which prevent normal semen deposition in the vagina.

Controlled ovarian stimulation appears to improve the chance of pregnancy and should be considered at an early stage for AIH [49], although the risk of multiple pregnancy should be minimised wherever possible. When dealing with male factor infertility, case selection is vitally important as poor results are usually obtained with reduced sperm motility [50]. Furthermore, the pretreatment of sperm with pentoxifylline can be particularly beneficial in cases in which the sperm show an impaired rate of acrosome reaction in response to a challenge by the ionophore A23187 [51].

Donated semen

The term donor insemination (DI) is preferred over the previous acronym for artificial insemination by donor (AID). The historical, clinical, technical, and ethical aspects have recently been reviewed [52]. The indications for DI include untreatable azoospermia (e.g. Klinefelter's syndrome, absent vasa, spermatogenic failure, epididymal obstructions), severe oligo/asthenospermic states and for genetic reasons (serious autosomal dominant conditions such as Huntington's disease, dystrophia myotonica, etc). However, the development of techniques such as intracytoplasmic sperm injection (ICSI), which enable the achievement of fertilisation with the male partner's sperm in cases with even the most severe impairment of spermatogenesis [53], will inevitably mean a shift in the referral patterns to DI programmes.

Because of the risk of transmission of human immunodeficiency virus (HIV) infection by the use of donated semen from an infected donor [54], accepted semen specimens in most countries are now frozen [55] and quarantined for 6 months, and can be released for clinical use only after a repeat screen of the donor (serological tests for syphilis, hepatitis B, hepatitis C and HIV) has proven negative [56]. Although not mandatory in some countries, it is generally considered to be no longer ethical to use fresh semen for DI treatments because of the risk of infection. In order to facilitate appropriate matching between donor and recipient, specific donor characteristics should be recorded including height, stature/body build, eye colour, hair colour, skin complexion, race/ethnic background and aspects of social history. However, information should be non-identifying and complete confidentiality must be maintained between donor and recipient. Notwithstanding these comments, restricted approval may be considered by some ethics committees for known-donor insemination, for example brother-for-brother donation in certain circumstances.

The cryopreservation of donated semen requires the use of an appropriate cryoprotectant to maximise the viability of thawed spermatozoa. Several cryoprotective media have been described but a particularly good one [57] contains 15% glycerol in a buffered solution of sodium citrate with glucose, fructose and streptomycin. The diluted semen is drawn into 0.5 ml coloured plastic straws which are preplugged at one end and then sealed with a coloured cement compound. The straws are labelled, grouped in plastic goblets with the cement ends uppermost and frozen using liquid nitrogen, with the coding system for the straws being based on their label, colour, position within the cryopreservation dewar and the particular dewar in which they are stored. Matching errors should not occur. An inexpensive hand-freezing technique, which involves precooling followed by slow freezing over nitrogen vapour prior to plunging into liquid nitrogen, may often suffice. Alternatively the straws may be frozen in a programmable freezer involving controlled rates of cooling (ranging from −1°C/min to −10°C/min down to −30°C, or even −80°C where it is held for 30 min prior to plunging into liquid nitrogen at −196°C within the storage dewar).

Inseminations are usually performed during the periovulatory phase when fertile cervical mucus is demonstrated (score≥6/12). The score is maximal just prior to the LH surge, after which it falls rapidly. Generally two or three inseminations are carried out. Care should be taken to use the thawed sample as quickly as possible to minimise deterioration, and so the appropriate straw may need to be transferred from the laboratory dewar to a smaller dewar or thermos flask within the clinic. When required the straw is removed from liquid nitrogen and allowed to thaw on an aseptic area on the workbench at room temperature. The cement end of the straw is then cut off and the straw placed in an insemination gun, covered by a sterile sheath and the tip of the instrument is inserted 1–2 cm into the cervix following its exposure using a bivalve vaginal speculum with the patient in the dorsal position. The semen is injected slowly with the speculum lightly gripping the cervix where it remains for approximately 2 min. After the instruments are withdrawn the patient remains recumbent for 15–20 min and may then leave the clinic, usually without restrictions being placed on subsequent activities, such as work or sport.

Live birth rates following insemination are usually disappointing, as illustrated in Table 60.2 by the total experience in the UK for 1991 and 1992. The data was collected by the Human Fertilisation and Embryology Authority in accordance with the Human Fertilisation and Embryology Act (1990) which made the notification of all treatments mandatory. Furthermore, a clear relationship between the chance of pregnancy and the woman's age can be seen, with the rates for older women being very poor. The results can usually be improved quite dramatically by the use of spermatozoa washed through Percoll and intrauterine insemination performed close to ovulation as determined

Table 60.2 Live birth rates following *in vitro* fertilisation (IVF) or donor insemination (DI) treatment in the UK for 1991 and 1992, according to the age of the woman being treated.

Maternal age (years)	IVF		DI	
	Cycles	Live birth rate (%)	Cycles	Live birth rate (%)
<25	278	12.6	1296	7.1
25–29	3539	18.1	9157	5.6
30–34	9365	16.3	14104	5.4
35–39	8142	12.5	8400	4.1
40–44	2645	5.4	2271	1.8
≥45	210	2.4	97	1.0
Total	24179	14.0	35325	4.9

Source: 2nd and 3rd Annual Reports of the Human Fertilisation and Embryology Authority.

by the accurate identification of the endogenous LH surge [58].

Infertility surgery

Significant tubal disorders causing disturbances of the ovum pick-up mechanism or ovum transport may be present in up to 35% of infertility cases. Almost half will demonstrate complete occlusions, these being within the distal region causing fimbrial agglutination or hydrosalpinges, or in the proximal regions as a consequence of intratubal cornual obstruction or intramural isthmic obstructions due to the enigmatic condition denoted as salpingitis isthmica nodosum. Partial occlusions and limited tubal mobility usually result from peritubal adhesions. The underlying cause of most cases of pelvic disease has, in the past, been ascribed to ascending infections of the genital tract with sexually transmitted organisms such as *Neisseria gonorrhoea* and *Chlamydia trachomatis* being strongly implicated. However, the evidence remains indirect and circumstantial. The pathogenesis appears clearer for many cases arising as a consequence of ascending non-specific infections following complicated parturition, early pregnancy losses and in association with intrauterine contraceptive devices, particularly when inserted in nulligravid women. Other pelvic pathologies such as ectopic pregnancy, endometriosis and ruptured appendix cause significant secondary infertility, and even *Mycobacterium tuberculosis* and *Actinomyces israellii* have to be considered within population subsets.

The underlying aetiology should be considered in all cases of recognised pelvic disease as it is imperative to control the process prior to definitive surgery or other infertility treatments. In a disturbingly large proportion of cases, the only factor related to pelvic adhesions is past pelvic surgery performed for incidental reasons such as ovarian cystectomy, ventrosuspension, unruptured appendicectomy and paratubal cystectomy. Fortunately, such operations are performed less frequently nowadays as laparoscopic and ultrasound-directed techniques have evolved. It appears that the main offending factors in the past have been glove powder or starch irritation combined with abrasive packing techniques.

Infertility surgery has a long history but the results were universally disappointing prior to the last decade. Recent advances relate to improved understanding of the pathogenesis of pelvic adhesions and the adoption of principles of microsurgery [59]. These principles include the use of an atraumatic technique and non-reactive suture material, careful attention to haemostasis, frequent irrigation of tissues during procedures and careful washout on completion to remove fibrinous clots. Pathological tissue must be completely excised, tissue dissection is preferably by fine needle-point electrocautery or laser coagulation techniques, and bipolar electrocoagulation is required for fine vessels within and near the Fallopian tubes. Packing should be performed with moist packs only, using a non-abrasive placement technique. Magnification, preferably with a free-standing operating microscope which allows foot-controlled zoom to at least ×25, enables the use of delicate instruments and fine sutures with the precise alignment and apposition of tissue planes, and serosal reperitonealisation using free grafts if necessary. The parietal peritoneum should be everted during closure to avoid omental adhesions to the anterior abdominal wall. Individual preferences may also include the use of parenteral prophylactic antibiotics, parenteral or pelvic steroids and high-molecular-weight dextran left within the cavity on completion to minimise postoperative pelvic adhesion formation.

The current trend to laparoscopic surgery within the female pelvis already appears to be associated with a reduction in the problem of postoperative adhesions.

Myomectomy and metroplasty

Submucous fibroids, and sometimes intramural or subserous fibroids, if causing distortion of the uterine cavity or impinging on the fallopian tubes, require excision. So too do uterine synechiae and sometimes a uterine septum if indicated by a past history of recurrent pregnancy losses. Currently, an increasing proportion of such cases are proving amenable to hysteroscopically directed surgical methods using laser or diathermy resection [60]. Large intramural and subserosal fibroids are now generally resectable at laparoscopy. These endoscopic microsurgical operations should largely replace the coarse laparotomy procedures of the past with improved results and fewer complications in both short- and long-term outcomes.

Reversal of sterilisation

For re-anastomotic reversals of female sterilisation, the patency rate is better than 90% and more than 60% of women have a successful pregnancy. The results are much better for those whose final tube length is >50 mm and particularly if the ampullary/infundibular end is preserved. The prognosis for reversal is best for clip sterilisations and less so for Silastic rings, mid-tubal resections, tubal diathermy, fimbriectomy and distal salpingectomy, in descending order. Many would consider the latter two, or even three, procedures unsuitable for reversal, regarding IVF as the better option. Ectopic pregnancies are relatively low after successful reversals, being around 2%.

Post-inflammatory tubal damage

Microsurgical tubal reconstructions performed for inflammatory disorders provide pregnancy rates of 20–40% for salpingostomy, 30% for fimbrioplasty, 40% for cornual implantation, 45% for salpingolysis and up to 60% for discrete resection/re-anastomoses. When compared with conventional surgery, microsurgery confers only marginal benefits in pregnancy rates for much of this group (although markedly better for cornual implantation and discrete re-anastomoses) and this relates to the wider extent of the damage inflicted by the underlying cause [61]. The ectopic pregnancy rate following surgery for inflammatory damage is variably reported as 5–20%.

Recently, operative laparoscopy has been evolving as an increasingly viable alternative to laparotomy procedures in certain pelvic conditions, including tubal pregnancy, salpingolysis, fimbrioplasty and salpingostomy [62].

Preparation for IVF-related procedures

When ovum aspiration was universally performed by laparoscopic techniques, benefits were often obtained by preliminary pelvic surgery with extensive adhesiolysis and partial omentectomy to free the ovaries, ventrosuspend the uterus and plicate the ovarian ligaments. Subsequent laparoscopic follicle aspiration was often improved or simply made possible [63]. However, most follicle aspirations are nowadays performed by a transvaginal ultrasound-directed method and it is preferable to leave the ovaries in the pouch of Douglas, obviating the need for previous surgery.

In recent days, preliminary pelvic surgery has once again been considered to enable tethered or obstructed fallopian tubes to be made accessible for GIFT or other tubal transfer procedures. However, the results indicate that an inordinately high ectopic pregnancy rate may occur [64]. Of greater interest has been the development of preliminary pelvic surgery in preparation for IVF. It would appear to be beneficial to clear endometriotic lesions as much as possible, at least to reduce the grading down to a mild degree, as the results of IVF are inversely related to the severity of the pelvic endometriosis. Endometriomas and other endometriotic lesions can be excised laparoscopically using simple excision techniques in association with electrosurgical ablation or laser coagulation. Other considerations include laparoscopic salpingostomy or even salpingectomy in the treatment of hydrosalpinges, and ovarian drilling for polcystic ovaries associated with significant hormonal disturbance—in particular, raised androgen and LH levels.

Male microsurgery (see also Chapter 50)

The introduction of microsurgical techniques on the male genital tract has led to an improved prognosis for vasectomy reversal and other discrete obstructions of the vas [65]. Some cases of epididymal obstruction, previously not possible to consider, are now amenable to microsurgery [66]. The prognosis for patency with sperm in the ejaculate after vasovasostomy is 80% or better, but for vasoepididymostomy it is dependent upon the level (caput, body or caudal region) and ranges from only 25 to 50%. However, the likelihood of pregnancy is reduced if anti-sperm antibodies have formed, if the sterilisation was long-standing, or if long segments of vas/epididymis were missing or bypassed as spermatozoa from the caput have reduced fertilising capacity.

Of greater potential benefit is the combination of microsurgical exploration of the epididymis with IVF. Male surgery performed in IVF theatres enables the identification of the appropriate region of the epididymis where motile spermatozoa can be identified, enabling the surgeon to better select the site of anastomosis. It also provides the opportunity for IVF in a combined procedure involving subsequent ovum aspiration from the female partner, fertilisation of the recovered oocytes and subsequent transfer of embryos. In all such cases it is now recommended that sperm be recovered from the testis or the epididymis and cryopreserved for potential subsequent IVF using the ICSI technique [67,68]. Successful pregnancies have now been reported from high epididymal recoveries of spermatozoa from men with absent vasa [69] or epididymal obstruction [70], and even from cases of significant spermatogenic failure.

In vitro fertilisation

Historical aspects

Although successful embryo transfers were described in the rabbit a century ago, the process of IVF has a much shorter history with the first mammalian success producing live

offspring being reported in 1959, again achieved in rabbits [71]. Interestingly, the rabbit model was not ideal with respect to spermatozoal capacitation *in vitro*, but this posed less of a problem than several other mammalian laboratory species where IVF was subsequently achieved. IVF has now been reported for a wide range of mammals, including non-human primates and domestic animals. Human IVF has proven to be relatively simple and is based on the mouse model initially described by Whittingham [72].

Crude attempts to achieve human IVF had been undertaken during the 1940s and 1950s, but it is unlikely that normal cleaving embryos were generated prior to the combined efforts of Robert Edwards (physiologist and embryologist) and the late Patrick Steptoe (gynaecologist), with contributions by Barry Bavister and the late Jean Purdy [73]. Edwards had earlier studied IVF in oocytes derived from surgical specimens of ovary and subsequently matured *in vitro*. The morphological quality of embryos was superior when preovulatory oocytes were aspirated from mature follicles at laparoscopy following stimulation with hMG and fertilised *in vitro* in a modified Tyrode's solution. They reported the first human IVF pregnancy in 1976 [74], but it proved to be an ectopic in the proximal segment of the distally occluded Fallopian tube. Interestingly, the team subsequently abandoned stimulated cycles as an ongoing pregnancy proved elusive and it was considered that such cycles were unfavourable for implantation.

The first successful pregnancies were achieved in a series of 32 cycles which reached the stage of embryo transfer (ET) after monitoring natural, unstimulated follicle development with a sensitive immunobioassay for LH performed on urine [75]. There were four pregnancies in that series and Louise Brown, a healthy female born in July 1978, became the first IVF infant [76]. A healthy male was also delivered a few months later but two other pregnancies miscarried, one in the first trimester shown to have triploidy, and another in the second trimester shown to have an inherited chromosomal anomaly [77]. The next team to report success was from Australia where IVF had been studied for almost a decade, and again this was achieved from a monitored natural cycle. However, such natural cycle pregnancies proved relatively elusive and subsequent successes were generally reported from stimulated cycles. By 1983 clinics were present in many countries around the world but most were reporting sporadic successes only amounting to a total of around 50 infants. Over the next 5 years there was a marked proliferation of service clinics and research activity leading to fairly consistent reporting of pregnancy rates in the range of 12–25%, an example of which is given in Table 60.2 from the data gathered in the UK by the Human Fertilisation and Embryology Authority.

Ovarian stimulation

The earliest workers in human IVF used hMG stimulation in order to generate several ovarian follicles when oocyte recovery techniques were relatively crude, and to counter the problems of limited fertilisation and limited developmental potential of oocytes. However, they recognized that luteal phases were shortened and the degree was directly related to the output of urinary oestrogens during the follicular phase [75]. Subsequently, when a rapid immunobioassay (Hi-Gonavis; Mochida Pharmaceutical Co., Japan) became available for LH/β-hCG detection, natural cycles were monitored and the single oocyte recovered, where possible. The first IVF pregnancies were achieved from natural cycles but the method was seen to have major limitations. These included the expense and inconvenience of prolonged hospitalisation for 8-hourly monitoring to detect the commencement of LH surge, the frustration of prolonged monitoring of disordered cycles, the difficulty of laparoscopic aspiration if the follicle was inconveniently located and inaccessible due to underlying pelvic pathology, and the need to access theatres outside a routine daytime schedule. Therefore, stimulated cycles were seen to be a requirement if IVF was to be adopted into clinical service.

The first clinic to report success with stimulated cycles [78] utilised clomiphene citrate alone for stimulation and hCG 5000 iu for the ovulation trigger. Oocytes were recovered 33–35 hours later and no luteal support was provided. Subsequently others, particularly in the USA, reported success using hMG alone for stimulation [79], triggering ovulation with hCG 10 000 iu and giving luteal support routinely in the form of progesterone 25–50 mg/day i.m. The latter was continued throughout the first trimester if pregnancy ensued. By 1986 when many IVF clinics were established worldwide, one of the most popular stimulation regimens in use combined clomiphene citrate with hMG. Generally, 50 mg clomiphene citrate was given b.d. day 2–6 or 5–9 and hMG ampoules (one to three) were given beginning a day or two after the clomiphene was commenced. Cancellation rates due to poor or inappropriate responses and premature LH surges were around 20%.

Marked improvements in IVF results have recently ensued from the diminishing use of clomiphene and the increasing use of GnRH analogues such as buserelin (Suprefact, Hoechst Laboratories) and leuprolide acetate (Lucrin, Abbott Laboratories), largely as a result of the blocking of an endogenous LH surge enabling ovarian stimulation to be continued longer and the follicles achieving a greater maturity as discussed in Chapter 48. The changing trend in ovarian stimulation regimens over the years is clearly shown in Fig. 60.2, which shows data collected on all pregnancies

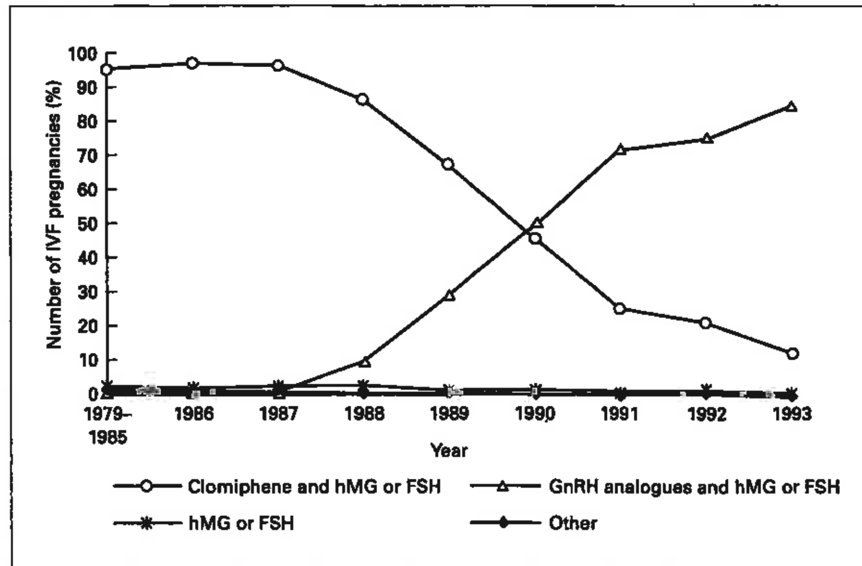

Fig. 60.2 The use of different stimulation regimens used for *in vitro* fertilisation (IVF) pregnancies achieved in Australia during 1979–93 [111].

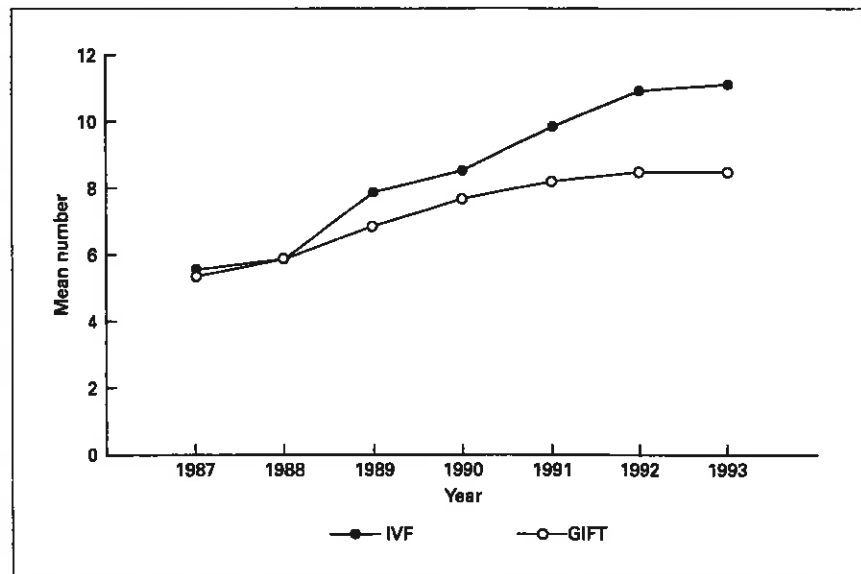

Fig. 60.3 The number of oocytes collected for *in vitro* fertilisation (IVF) and gamete intra-Fallopian transfer (GIFT) pregnancies in Australia during 1987–93 [111].

generated in Australia between 1979 and 1993. Occurring in tandem with the change in fashion in ovarian stimulation protocols has been an increase in the number of oocytes collected, as shown in Fig. 60.3.

Oocyte collection

Traditionally, oocyte recovery developed as a laparoscopic procedure but has increasingly become replaced by ultrasound-directed techniques, particularly the transvaginal approach. The optimisation of oocyte recovery has been shown to depend upon three main aspects [63]:

1 timing the recovery following LH surge or hCG induction and inducing the surge at the appropriate stage of follicle maturation;

2 the instrumentation and techniques applied for aspiration of the oocytes from follicles; and

3 accessibility of the ovaries for aspiration.

With respect to timing, the LH surge or hCG trigger should occur in cycles down-regulated with GnRH analogues when three or more follicles are 20 mm in diameter. Thereafter, follicles are aspirated 36 ± 2 hours after initiation of the LH surge or hCG trigger. Oocytes aspirated earlier than 34 hours may benefit by compensatory *in vitro* culture prior

to insemination but embryo quality is poor and pregnancy rates are low if oocytes are recovered 4 or more hours earlier than optimal. Oocytes collected up to 4 hours after the optimal time remain equally suitable but the risk of spontaneous oocyte release increases although this appears to be <10% up to 42 hours in GnRH analogue cycles.

The matters of instrumentation and accessibility are considered separately for laparoscopic and ultrasound-directed recoveries.

Laparoscopic recovery

Laparoscopy requires general anaesthesia and endotracheal intubation. Access to follicles may be restricted by pelvic adhesions hence in the past preliminary pelvic adhesiolysis, ventrosuspension and plication of the ovarian ligaments has been recommended. Whilst this has significantly improved laparoscopic access, it may prejudice transvaginal access and hence is no longer encouraged.

A wide range of single- and double-lumen needles are in common use but the latter are proving increasingly popular as they enable follicle flushing. Those which enable a fine-spray flush with a continuous flow-through system such as the PIVET-Cook Laparoscopic/Ultrasound Double Lumen Ovum Pickup Needle [William A Cook, Australia] provide optimal oocyte recovery rates, being >90% of mature follicles. The technique involves needle puncture of the follicle under direct laparoscopic vision and aspiration of the contents into a 16-ml polystyrene test tube. Whilst the contents are being examined under stereomicroscopy by the embryologist in an adjacent IVF laboratory, the follicle is flushed with Hepes-buffered medium prior to moving to the next follicle.

The postoperative recovery of women after laparoscopy is sometimes uncomfortable due to the anaesthetic drugs, the laparoscopic wounds and residual abdominal gas. Serious complications among IVF cases, including deaths, are usually anaesthetic-related and occasionally due to the inadvertent puncture of bowel, bladder or vascular structures.

Ultrasound-directed recovery

The first reports using ultrasound guidance for follicle aspirations were reported from Scandinavia [80] and described a transcutaneous transvesical method. Subsequently, a transurethral method was explored briefly and finally the transvaginal method has found popular acceptance [81]. The optimisation of transvaginal ultrasound-directed aspiration requires the following [82].

1 Minimal anaesthesia, for example propofol intravenous anaesthetic or premedication combined with local anaesthesia.
2 Application of a pressure band to the lower abdomen to stabilise the ovaries and prevent them slipping away during attempts at penetration.
3 Use of very sharp needles with echo-enhanced tips which enable an efficient follicle flushing technique. The aforementioned PIVET-Cook needles were designed specifically for the purpose and are ideal.
4 Follicle aspiration and flushing is performed as previously described for laparoscopic access. It is ideal to have the theatre and IVF laboratory combined or adjacent. During follicle flushing, the follicle is only partially refilled so the flush and aspiration procedures proceed simultaneously. This requires a high-pressure fine jet to avoid 'short-circuiting' the follicle and again the PIVET-Cook needles are suitably designed.
5 The control of flow through the aspiration needles is governed by Poiseuille's Law, hence aspiration pressures require adjustment depending upon needle length (factor of ×8, e.g. 35-cm 16-FG needle requires −180 mmHg whilst 25-cm needle requires −100 mmHg) and needle diameter (inversely related to fourth power of the radius).
6 High-resolution ultrasound image is required, for example General Electric electronic phased array sector scanner with 5.0 MHz vaginal probe and needle guide is widely and effectively used.
7 The vaginal probe requires a coupling medium, such as culture medium placed in the vagina at the beginning of the procedure after saline wash-out, to maximise the contact and quality of the picture. Sterilising fluids are avoided because of possible toxic effects upon the oocytes, hence it is imperative to exclude the presence of vaginal pathogens earlier in the treatment cycle.

Gamete and embryo culture

Ideally, an IVF unit should comprise two 'embryology' laboratories—one dealing with culture-media preparation, semenology, cryopreservation, the cleaning and sterilisation of equipment, and quality-control aspects; the other as a dedicated human IVF laboratory as part of the theatre complex, which is maintained as an aseptic, quiet and low-traffic area.

Gamete preparation

From follicle aspirates and flushes, the cumulus–corona–oocyte complex is recognised as a silvery gelatinous mass which can be graded according to the overall size of the mass, number and degree of dispersal of cumulus cells, and tightness or density of the coronal cells. They are removed from the follicle flushing medium and washed twice before placing in culture medium supplemented with serum or an equivalent protein preparation. This is usually done in culture

tubes or multiwell dishes. It is crucial to avoid temperature reductions as the meiotic spindles can be irreversibly damaged on cooling [83,84]. Routinely, oocytes are preincubated for 4–6 hours prior to insemination but longer periods may be required, particularly if the oocytes are immature.

Spermatozoal preparations are performed routinely by the swim-up method [114] for normospermic semen samples (Fig. 60.4). However, oligozoospermic and asthenospermic semen require the use of a discontinuous gradient separation using Percoll to achieve pure motile spermatozoal samples which are free of debris and other cells, as shown in Fig. 60.5. Current research in this area is evaluating methods of preparing spermatozoa which remove reactive oxygen species [85] (i.e. free hydroxyl and oxygen radicals and non-radical hydroperoxides) which damage cell membranes and sperm function. In this regard renewed interest is being paid to vitamin C and α-tocopherol, the active ingredient of vitamin E. In selected cases, sperm motility can be boosted by adding a cyclic adenosine monophosphate (cAMP) inhibitor such as pentoxifylline which will also improve the fertilisation rate [51].

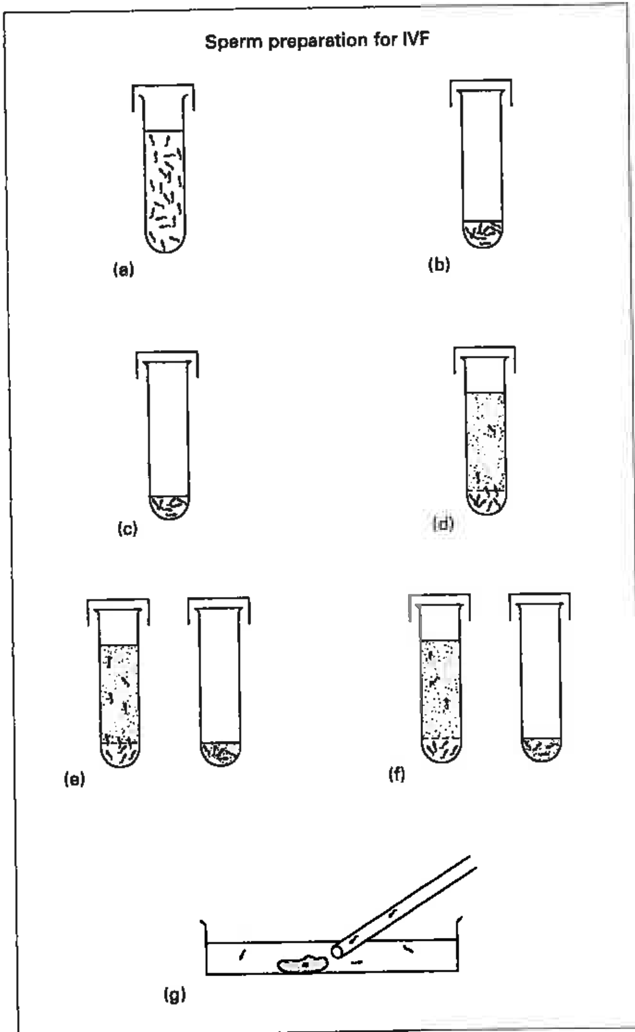

Fig. 60.4 The technique of sperm preparation by the swim-up method (with permission from Plenum Press) [86]. (a) 0.4 ml of whole semen diluted to 5 ml with medium. (b) Sample centrifuged 2–3 times at 200 *g* × 10 min. (c) Pellet resuspended in 0.5 ml. (d) Sample overlaid with 4.5 ml medium. (e) Top of supernatant containing motile sperm harvested. (f) Remaining supernatant removed if sperm yield poor. (g) 0.5–2.0 × 10^5 spermatozoa added per millilitre of medium containing the oocyte–cumulus mass.

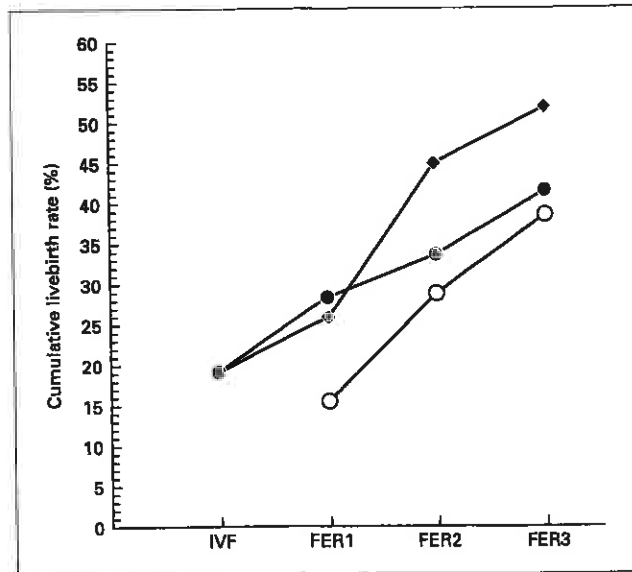

Fig. 60.5 The cumulative live birth rate in parents for the initial *in vitro* fertilisation (IVF) cycle followed by the replacement of cryopreserved embryos on one (FER1), two (FER2) and three (FER3) occasions [112]. Cases had remaining IVF embryos cryopreserved at the pronucleate (●—●) or early cleavage stage (◆—◆), or all embryos cryopreserved at the pronucleate stage because of the risk of ovarian hyperstimulation (○—○).

Fertilisation

Spermatozoa are added to single oocytes at a concentration of 50–100 000/ml. Generally this is performed in tubes, although many units prefer to incubate in microdroplets (25–50 μl) under oil and this is beneficial when sperm numbers are low. Very small volumes can be achieved (5–10 μl) within straws. Incubation conditions must be stringently controlled [86] so the culture environment is humidified, held constant at 37°C, and gassed (ideally with 5% CO_2: 5% O_2: 90% N_2 although 5% CO_2 in air appears to suffice) to maintain the culture medium at pH 7.3. The culture medium should have an osmolarity around 285 mOsmol/l and be prepared from high-grade chemicals dissolved in highly purified water [87]. Serum additions also require stringent handling and preparation conditions, including complement deactivation.

It should satisfy strict quality-control assessment such as supporting mouse IVF or mouse embryo culture from one-cell to expanded blastocysts at a rate ≥85%.

The fertilisation process should be checked at 14–18 hours after insemination to determine the number of pronuclei within the oocyte. This involves microdissection to remove the coronal coat of cells (Fig. 60.6). The cumulus cells should have fully dispersed from hyaluronidase released from the spermatozoal acrosome cap. The check for pronuclei is important as 5–8% of seemingly normal preovulatory oocytes will contain more than two (the proportion may be much higher in poor-grade oocytes), implying polyspermy, and 1–2% may only have one, implying parthenogenetic activation. Subsequently, multipronucleate oocytes cleave rapidly into embryos which are usually, and deceptively, of the highest morphological grades, although their chromosomal abnormalities have been well defined [88]. Such oocytes should always be identified and excluded from transfer and this constitutes one of the limitations of the GIFT procedure. In addition, 25–30% of oocytes will fail to fertilise. Many will fertilise after re-insemination but resultant embryos rarely, if ever, become successfully implanting embryos resulting in live infants.

After the check for pronuclei, oocytes can be returned to short-term culture in simple medium, but some workers prefer a more complex medium such as Ham's F10 or MEM (minimal essential medium) as these have been shown to be superior for culturing through to blastocysts [89]. Human serum will also support embryo culture but current research is directed towards coculture methods, for example with endometrial or tubal cells. Embryos can be graded and scored according to morphological features, for example the regularity of blastomeres, degree of fragmentation and clarity, or granularity of the cytoplasm, as well as the cleavage times. Generally, embryos are transferred around 44–48 hours after insemination when most are at the four-cell stage.

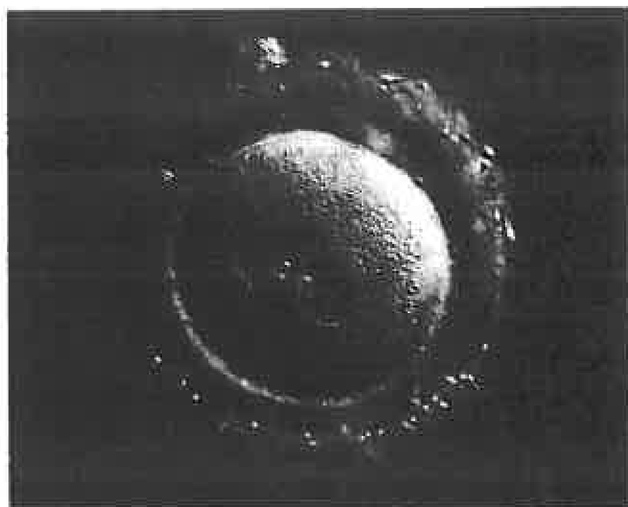

Fig. 60.6 Pronuclear oocyte after microdissection of coronal cells 14 hours after insemination.

Embryo cryopreservation

The cryopreservation and storage of human embryos remaining after an IVF cycle has proved invaluable in giving patients the opportunity to become pregnant by the subsequent replacement of thawed embryos, without the need for repeat ovarian stimulation and oocyte recovery, as shown in Fig. 60.5. Other important clinical uses of cryopreservation include the elective freezing of all embryos in cases at risk of ovarian hyperstimulation syndrome (OHSS), and the quarantine of fertilised donated oocytes to minimise the risk of infection to the recipient [90].

Cryopreservation can be done at a number of stages of embryo development using a variety of cryoprotective agents [91]. Briefly, embryos can be frozen at the pronucleate stage using propanediol, two-cell to eight-cell using propanediol or dimethylsulphoxide, or at the blastocyst stage using glycerol. Survival rates of 60–70% per embryo after thawing are commonly reported, with the initial quality of the embryo appearing to be important in determining survival [92].

The transfer of embryos is usually done in either a natural cycle, where the transfer is timed relative to the endogenous LH surge, or in cycles in which the endometrium has been prepared with exogenous oestrogen and progestogen. An example of a typical replacement cycle is shown in Fig. 60.7.

The cryopreservation of human oocytes would be extremely beneficial in an assisted conception programme. The gametes could then be fertilised upon thawing, circumventing the ethical dilemma posed when embryos are in storage and the couple separate. Also, the cryopreservation of oocytes from women, prior to undergoing chemotherapy or radiotherapy, would be helpful in maintaining their reproductive potential even though the gonads would sustain irreversible damage. Whilst advances are being made in the development of effective techniques [93], it will be a while before oocyte cryopreservation is available routinely.

Ovarian hyperstimulation syndrome

Apart from the problem of multiple pregnancies, OHSS is the main serious and life-threatening complication of ovarian stimulation. It is, therefore, an iatrogenic disorder and was first reported in 1964 in a woman who developed a wide spectrum of clinical symptoms and signs after ovulation induction. A useful classification put forward by the World Health Organization Scientific Group [94] describes three grades of severity. In its severe form there is massive ovarian enlargement, ascites, pleural effusions, haemoconcentration,

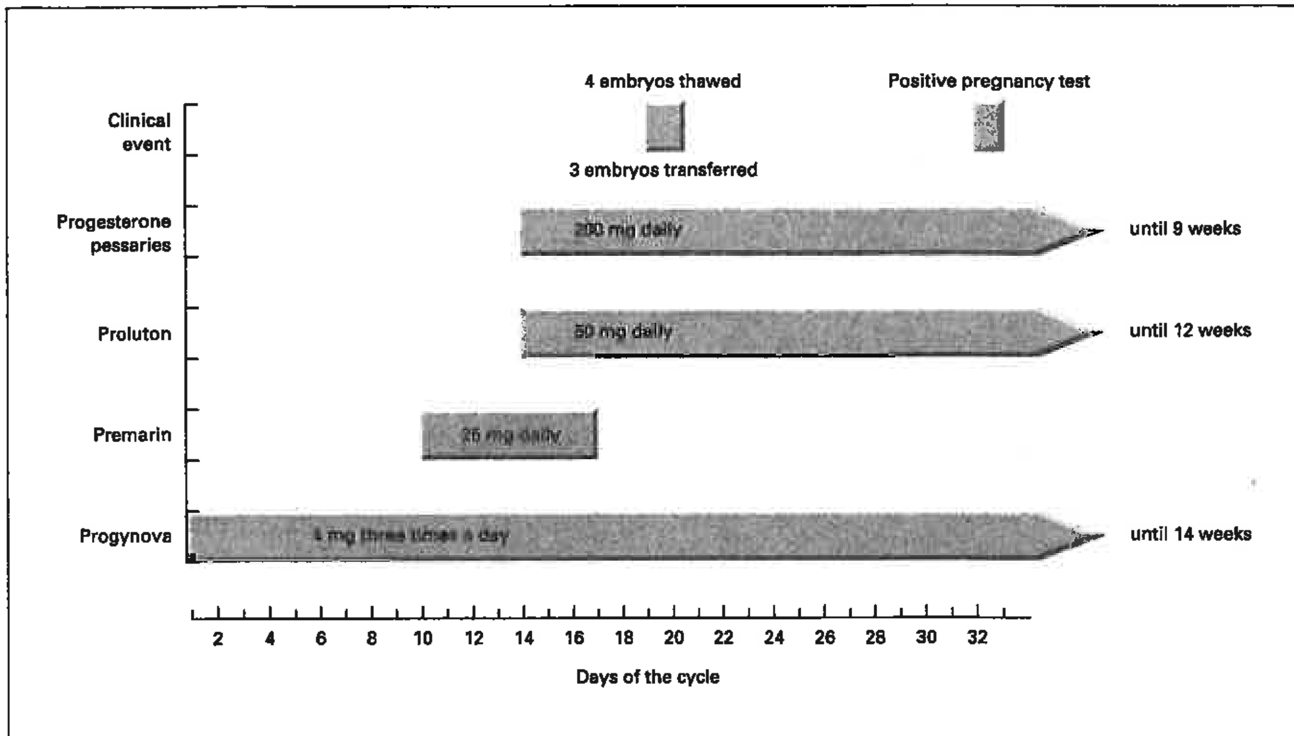

Fig. 60.7 A typical hormone-replacement cycle resulting in a viable pregnancy following the transfer of cryopreserved embryos.

oliguria, electrolyte imbalance and hypercoagulability. The main symptoms are nausea, vomiting, abdominal pain and distension, but the condition can be further complicated by rare features such as deep-vein thrombosis, cerebrovascular accidents, chronic liver disease and torsion of the ovary. To date, the pathophysiological mechanisms have not been elucidated and studies have concentrated on plasma renin activity, changes in aldosterone and the renin–angiotensin cascade [95].

OHSS requiring hospital admission is rare after clomiphene citrate alone but occurs in 1.5–3% of cycles where hMG is used. Younger women with polycystic ovary syndrome and highly responsive ovaries are most prone (conversely those women requiring very high doses of hMG are least prone) and the rates may be a little higher in cycles using GnRH analogues. Strategies used within IVF programmes to minimise the risk of symptomatic OHSS in women showing an exaggerated response to ovarian stimulation include the cancelling of the treatment cycle, the withholding of hCG as a luteal support, and the cryopreservation of all embryos generated, with thawing and replacement taking place in a subsequent cycle after the hyperstimulation has subsided [96].

Oocyte micromanipulation

Micromanipulation procedures on oocytes are undertaken to enhance fertilisation in cases of severe male factor infertility. Several techniques have been described, as shown in Fig. 60.8. The main difficulty was originally thought to be the crossing of the zona pellucida of the oocyte, and so a modification of the IVF protocol was introduced called partial zona dissection [97], in which a slit was cut through the zona with a sharp glass needle. This then acted as a gate through which the sperm could swim to gain access to the perivitelline space and interact directly with the vitelline membrane. Unfortunately, many patients still had no fertilisation, with the sperm often being unable to pass through the slit. Another difficulty was that the zona pellucida is the main block to polyspermy in the human, and so many of the oocytes became polyspermic. Subzonal insemination was then introduced [98] by which a controlled number of spermatozoa were injected directly into the perivitelline space to ensure that the zona pellucida had been bypassed. Polyspermy was still a problem, being related to the number of spermatozoa injected, but the main limitation was the persistently reduced fertilisation rate seen. This implies an inability of the spermatozoa to successfully cross the vitelline membrane. The introduction of intracytoplasmic sperm injection (ICSI) [99] appears to offer the best chance of achieving a pregnancy since excellent fertilisation and implantation rates are now being obtained by many centres

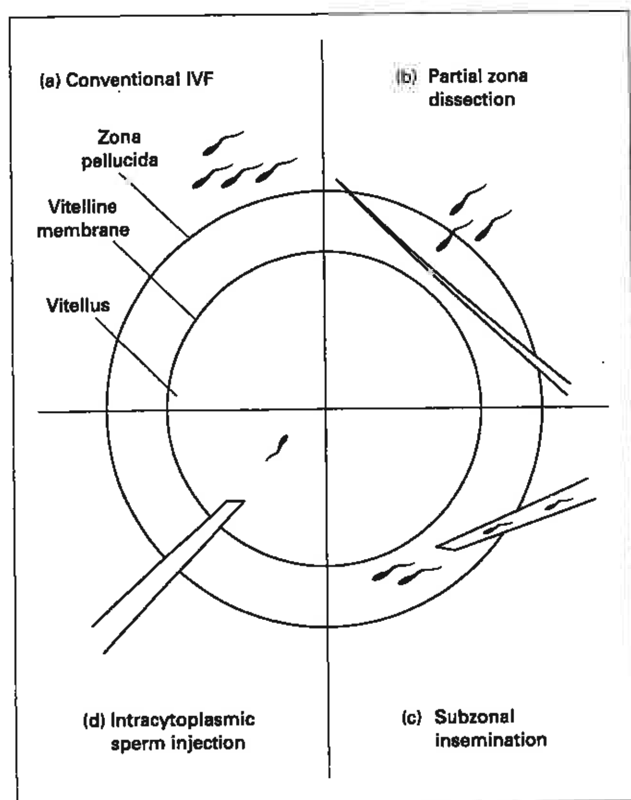

Fig. 60.8 The different *in vitro* fertilisation (IVF)-related strategies employed in the treatment of severe male factor infertility.

around the world. In this technique, a single spermatozoon is injected directly into the cytoplasm of the oocyte, bypassing both the zona pellucida and the vitelline membrane. The single sperm for ICSI can be obtained from ejaculate specimens in cases of severe oligoasthenozoospermia; epididymal aspirations for obstructive azoospermia and even testicular biopsy samples to tease spermatozoa from seminiferous tubules in cases of spermatogenic failure [115].

IVF-associated techniques

Gamete intrafallopian transfer

In many infertility clinics GIFT [100] has become established as the main procedure for non-tubal, non-male infertility. It is therefore a commonly used treatment for resistant cases of unexplained/poorly explained infertility, those with poor sperm–mucus interaction, those with pelvic endometriosis and cases of failed DI therapy. It is also a suitable option for those women likely to ovulate more than three oocytes during ovulation therapy and who should otherwise avoid conception in that cycle.

The ovarian stimulation regimen is the same as described for IVF and oocytes are recovered either by laparoscopy or by the transvaginal method. The choice depends upon surgeon preference and the proposed technique of transfer. The early workers relied on a mini-laparotomy procedure for oocyte transfer, but techniques were soon described facilitating laparoscopic tubal cannulation which is now the most commonly used method. More recently, transcervical cannulations have been explored. Up to four oocytes (usually two to each tube) are usually transferred but many clinics have reduced to three (usually all to one tube) without compromising pregnancy rates but significantly reducing the risk of high-order multiple pregnancies. Oocytes are loaded into a Teflon transfer cannula in 25 μl of medium along with 100 000 spermatozoa (male partners being required to produce their semen samples 2 hours prior)—also in 25 μl separated by a small air space. The gametes are deposited 40 mm into the Fallopian tube via the fimbrial end. If transcervical cannulation is performed, anaesthesia is not required and gametes are deposited approximately 40 mm from the cornual orifice. If GIFT is used for oligospermic infertility, higher numbers of spermatozoa are required but this is no longer recommended as blighted ovum pregnancies are increased [101].

GIFT patients need to consider the question of their supernumerary oocytes after transfer of their best three. The options for such supernumerary oocytes are to fertilise and cryopreserve them for the couple's own subsequent use, donate them for use by other infertile couples, donate them to an approved research program, or simply discard them. Supernumerary oocytes comprise those with poorer scores on grading and hence have reduced fertilisation potential [101]. Generally, it is younger women who respond sufficiently well to stimulation enabling ovum donation to be considered. Some clinics have established as stand-alone GIFT units and may consider the technique of intravaginal culture [102], enabling their patients to avail themselves of the services of a regional IVF unit for embryo cryopreservation rather than having to discard their supernumerary oocytes.

Intravaginal culture

The desire to obviate the need for an embryology laboratory led to the development of the technique of intravaginal culture [103]. The oocytes and embryos were placed into a tube containing culture medium, and the tube placed into the patient's vagina to be kept warm. The tube was then removed 48 hours later at the time of embryo transfer and the cleaved embryos selected and replaced into the uterus. After an initial following, the technique has not been used widely. The main limitations are: (i) that the only real saving is the price of an incubator since staff able to handle gametes and embryos are still required; and (ii) the omission of the check for pronuclei means that some oocytes will be

polyspermic but divide to form embryos of normal appearance and so be transferred.

Transport IVF

The establishment of a good IVF laboratory is of paramount importance in obtaining good pregnancy rates in an IVF programme. The transport of oocytes collected at a peripheral unit to a central specialised laboratory for basic IVF [104] or micromanipulation [105] has therefore proved extremely valuable in increasing the access of patients to treatment. This strategic use of limited resources is an excellent way of providing IVF and is to be encouraged in areas where the establishment of a full IVF unit is not feasible.

Ovum donation

Ovum donation resulting in a successful pregnancy was first reported in 1984 [106] and was applied to a woman with primary ovarian failure. The indications have since been extended and now include cases with incipient ovarian failure, those who respond poorly to ovarian stimulation, cases with serious genetic carrier states and occasionally cases whose ovaries are inaccessible, although this is uncommon now given the wide variety of techniques available to recover oocytes. Known or anonymous donors are required to provide the oocytes and these women must undergo full ovarian stimulation, monitoring and oocyte recovery. Not surprisingly, altruistic donors are hard to find because of the practical difficulties faced [107].

The best results reported from ovum donation involve tubal transfers performed in cycles established by a hormone-replacement regimen, for example oestradiol valerate and progesterone injections or pessaries. Pregnancy rates are generally reported in the range of 35–50% per transfer indicating that around 20% of such embryos may implant [108]. This could relate to the generally younger age of donors and the benefits of a hormonally controlled cycle. The latter view has led to the use by some clinics of GnRH suppression followed by hormone replacement in cycling women having either ovum donation or their own cryopreserved embryos transferred, with improved results. Pregnancy wastage following ovum donation is low, generally being reported as 20% or less.

Surrogacy

The issue of surrogacy remains controversial such that in some locations it has been legislated against. However, a good case can be made for the concept of altruistic IVF surrogacy [109] otherwise known as 'compassionate family surrogacy'. This embodies the following four criteria:

1 an appropriate medical indication, for example absent uterus, uterine malfunction such as Asherman's syndrome or a medical condition which contraindicates pregnancy;
2 embryos are generated from the gametes of the infertile couple (or a matched anonymous donor if indicated);
3 no surrogacy fee is paid (although the surrogate's treatment expenses may be covered by the infertile couple); and
4 a relative (usually a sister) or very close friend acts as surrogate.

Such altruistic IVF surrogacy arrangements appear to have entirely satisfactory outcomes and the concern over relinquishment does not appear to arise. Children should be informed from the earliest stages of the nature of their biological backgrounds and the assistance provided by their special aunt who carried them through pregnancy on their mother's behalf. In the main, the genetic parents will be required to adopt their own children because of the common law view that a woman who delivers a child is regarded as the mother and the absence of specific supportive legislation. However, there are now parts of the world (e.g. California) with specific legislation in place to allow the commissioning parents to be regarded as the legal parents in the first instance.

Ethical and legal considerations

IVF and related areas of assisted reproduction have generated unprecedented public interest in a medical area. Certainly there are broader social, ethical, legal, religious and sometimes political issues which arise apart from the complexity of technical issues. There are four broad areas of concern:
1 standards of laboratory and clinical practice;
2 accountability to the general community;
3 protecting the welfare of children born following assisted reproduction;
4 ownership of stored gametes and embryos.

Guidelines and regulations should assist to limit the complications (e.g. high-order multiple pregnancies, ovarian hyperstimulation syndrome and anaesthetic mortalities) and ensure clinics are providing the best possible service to infertile couples, within the current limitations of knowledge. In this latter context, the need for continuing research into all aspects, including fundamental physiology as well as clinical applications, must be acknowledged and pursued. Public accountability can be incorporated within a self-regulatory mechanism by ensuring an accurate and current data reporting system which is accessible. The welfare of children, and potential children, means careful control over the disposal of gametes, avoiding mixed embryo transfers which might confuse the genetic identity of children, respecting confidentiality of donors but enabling those children who become aware of a donor background to

have access to non-identifying information. Debates are current concerning access to identifying information and the question of respective responsibilities with respect to IVF surrogacy infants. Other concerns relate to ownership of stored embryos in the event of a couple's separation or death, and ensuing disputation arising over the use of these embryos. Such matters can only be resolved by specific legislation.

Conclusions

Procedures to assist reproduction have made a major impact in the area of infertility over the past decade and have been based upon wide-ranging advances in knowledge concerning reproductive physiology. This has occurred at an appropriate time as the fecundity of many industrialised communities has decreased markedly in recent years. In their turn, the procedures themselves have created the opportunity to consider providing services for the fertile population, for example in controlling genetic disease, in gamete and embryo storage for the preservation of fecundity and in new considerations for contraception. The field has excited considerable public interest and has implications for other areas of medicine such as the team approach to the management of individual cases, control by institutional ethics committees and other regulatory bodies, both voluntary and statutory. The main danger is the snowballing effect of a perceived need to introduce legislative controls, particularly in the area of embryo research, which may create an inhibitory or oppressive climate for further research [110]. Such legislation may effectively seal the current technology in its relatively inefficient state.

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