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Ovarian stimulation for assisted conception

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Introduction

Ovarian stimulation was initially introduced as a medical therapy for women who were clearly anovulatory evidenced by amenorrhoea or severe oligomenorrhoea. However, the role of ovarian stimulation currently has emerged as an integral treatment mode in different assisted conception procedures for a wide spectrum of infertility problems.

The spectrum of infertility

Involuntary infertility is a worldwide problem which causes a sense of personal failure as well as carrying a social stigma in many cultures. Infertile couples describe a sense of anguish, and often despair, when their desire to reproduce remains unfulfilled. Without contraception, 75% of couples would achieve pregnancy within 12 months, mostly within the first 4 months (Wilcox *et al.*, 1988). Thereafter the proportion conceiving is relatively low, and overall up to 15% of couples within the reproductive age range present for medical assessment, usually by 2 years of failed effort.

The aetiological factors underlying infertility are numerous and the predominant causes vary with geographical location, socio-economic factors and the changing face of health problems within different areas in given periods of time. For example, tuberculous disease of the genital tract is suspected to be the most prominent underlying condition in one recent study from Iran (Bahadori, 1986), whereas ovulatory dysfunction heads the list in an Australian report (Cox, 1975).

The broad categories of infertility recognized in industrialized societies are ovulatory dysfunction (25–45%), spermatozoal disorders (mostly unexplained; 20–35%), tubal disease (15–30%), pelvic endometriosis (10–15% as the attributed cause; up to 45% as an identified factor), poor sperm/mucus interaction (5–15%),

antispermatozoal antibodies (ASABs; 5–15%), and completely unexplained (5–10%). With comprehensive investigations conducted on both partners, most cases will reveal a multifactorial basis although often a single condition will appear to have predominant relevance over other identified factors.

Uncommon causes of infertility include genital tract anomalies such as congenital absence of a vital structure (e.g. Rokitansky–Küster–Haüser syndrome androgen insensitivity syndromes, congenital absence of vasa deferentia) as well as those caused by diethylstilboestrol exposure *in utero*, and intrauterine synechiae causing Asherman's syndrome. Sexual dysfunctions and ejaculatory disorders are occasional causes of infertility owing to failure of sperm deposition in the vagina.

Therapeutic prognosis

Prior to 1960, it appears that less 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 (Jeffcoate, 1975). Subsequently the prognosis improved with the introduction of clomiphene citrate (CC) and gonadotrophins (from human menopausal urine; hMG and human pituitary glands; hPG) for ovulation induction. This was associated with inspired discoveries and developments in understanding the hypothalamic–pituitary–ovarian axis leading to the current improved level of knowledge regarding events concerning folliculogenesis, oocyte release and luteal function in the ovarian cycle. hPG was subsequently withdrawn from clinical use because of the aetiological relationship with Creutzfeld–Jacob disease which subsequently developed in some treated women (see later).

Further significant advances during the 1960s and 1970s included the introduction of laparoscopy as a primary diagnostic tool; the development of sensitive, specific and eventually, rapid hormone assay systems; 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 successful treatment with bromocryptine; the establishment of donor semen banks using frozen straws; the development of microsurgery (initially on the female and subsequently on the male genital tract), advances in endoscopic operative procedures, and the detection of antibodies against gametes. Such advances improved the prognosis considerably and the understanding of human infertility sufficiently, to enable the introduction of techniques to assist human reproduction.

The integration of assisted reproductive methods with these 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 livebirth. The main limiting factors to the successful treatment of infertility are no longer technical but relate to 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.

Assisted conception procedures

Historical aspects

Although successful embryo transfers were described in the rabbit a century ago, the process of *in vitro* fertilization (IVF) has a much shorter history with the first mammalian success producing live offspring being reported in 1959, again achieved in rabbits (Chang, 1959). Interestingly, the rabbit model was not ideal with respect to spermatozoal capacitation *in vitro*, but this posed less problem with 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 current practice is based on the mouse model (Whittingham, 1968).

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 (Evans, Mukherjee & Schulman, 1980). 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 pre-ovulatory oocytes were aspirated from mature follicles at laparoscopy following stimulation with human menopausal gonadotrophine (hMG) and fertilized *in vitro* in a modified Tyrode's solution. They reported the first human IVF pregnancy in 1976 (Steptoe & Edwards, 1976), but it proved to be an ectopic in the proximal segment of a distally occluded Fallopian tube. The team subsequently abandoned stimulated cycles as an onging 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 immuno-bioassay for LH performed on urine (Edwards, Steptoe & Purdy, 1980). There were four pregnancies in that series

and Louise Brown, a healthy female born in July 1978, became the first IVF infant (Steptoe & Edwards, 1978). 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 (Steptoe, Edwards & Purdy, 1989). 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, natural cycle pregnancies proved to be relatively elusive and subsequent successes were derived from stimulated cycles. By 1983, IVF clinics were commenced in many countries but most were reporting sporadic successes only, amounting to the birth of around 50 infants. In the next 5 years clinics reported consistent pregnancy rates of 12–25%. However, worldwide reports from independent authorities published on data to 1987, showed the livebirth rate per IVF treatment cycle for those patients who reached the stage of oocyte recovery averaged only 9–10% (Yovich *et al.*, 1989).

During 1987–1992 improvements in methodology, increasing expertise and new knowledge in many aspects of reproductive medicine have led to higher rates of couples successfully completing their treatment cycles (>80%) and overall livebirth rates of 20–25% per cycle reaching the embryo transfer stage are becoming more common (Cohen, de Mouzon & Lancaster, 1993). By the end of 1992 it was estimated that there were in excess of 100 000 infants born as a result of IVF-related procedures worldwide. This achievement is the tangible consequence of advances in the science of human reproduction over the past 20 years. The new knowledge has also been applied effectively in a range of non-IVF treatments for infertility as well as opening the door to major advances in the management of genetic-based diseases, improved contraceptive methods and perhaps assisting the peoples of industrialized countries to sustain their population in a socio-economic climate causing reduced fecundity.

Physiological considerations

The ovarian stimulation protocols used in the various techniques of assisted conception are based on a clear understanding of existing knowledge in human reproduction, having evolved from major advances in knowledge of areas such as the hypothalamic–pituitary–ovarian axis and its relationship with apocrine events in the gonads and endometrium.

As advances in knowledge accrue, protocols can be adjusted accordingly. Reproductive medicine is advancing rapidly partly because of the establishment of dedicated clinics, with commitments to both service and research aspects. Such clinics are able to undertake fundamental research and develop new techniques.

Table 1. *Specific facilities required for the comprehensive management of infertility, preferably within a single unit functioning every day*

Consultation	• both partners
Counselling	• information • emotional support
Co-ordination	• senior nurse • tests/instructions/results
Laboratories	• andrology • embryology • cryopreservation • hormone assays
Ultrasound	• 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

Range of techniques

Modern treatments to assist conception can be categorized as specific, general or substitutive. Ideally, these modes are all carried out in a single unit structured to provide a comprehensive approach to infertility management (Table 1).

Specific treatments

Specific treatments include reconstructive pelvic and tubal microsurgery; the management of endocrine disorders including hyperprolactinaemia, hyper- and hypo-thyroidism, Cushing's disease and hypopituitarism; ovulation induction for discrete anovulatory disorders; and both medical and surgical treatments for pelvic endometriosis. These treatments are invariably followed by successful conception and pregnancy outcomes. The likelihood of success is dependent upon the relevance of the diagnosed disorder to the couple's infertility, the association

Table 2. *Optimal assessment prior to commencing infertility treatments*

1. Clinical examination	–	both partners
2. Routine tests	–	ECS and Pap smear
		Semen analysis
		ASABs–serum both partners
		–semen, cervical mucus
		Hep B and C, HIV screen both
3. Ovarian cycle	–	FSH, LH, Prolactin in early phase
	–	Androgen status in early phase
	–	Ultrasound in early phase
	–	E_2, P_4, LH in periovulatory phase
	–	Ultrasound in periovulatory phase
	–	Mucus score in preovulatory phase
	–	E_2, P_4 in mid-luteal phase
4. Post-coital test	–	8–12 h post coitus
		Immediate preovulatory phase
5. Laparoscopy	–	Pelvic assessment
	–	Tubal dye pertubation
6. Hysteroscopy	–	±Hysterosalpingogram
7. Counselling	–	Early introduction

Further specific investigations and management required following the detection of abnormal results in any of the above areas.

of additional factors, and the effectiveness of the specific treatment to correct the disorder and reverse any underlying damage to reproductive mechanisms. These comments highlight the need for the comprehensive assessment of both partners and the sperm/cervical mucus interface prior to, or in association with, any proposed treatment mode (Table 2).

General treatments

General treatments provide solutions to multifactorial and poorly explained infertility problems as well as those resistant to or unsuitable for specific treatments. They include *ovarian stimulation* of women who appear to be ovulatory and the use of a range of procedures involving *gamete manipulation* in order to enhance the chance of fertilization or bypass the fallopian tubes for embryo placement. Whilst there may be a dominant single condition indicating the need for such assistance, in many cases more than one factor can be identified and

Table 3. *Infertility treatments involving gamete manipulation*

Insemination	
DI	donor insemination
AIH	artificial insemination (husband)
IUI	intrauterine insemination
IVF-related	
GIFT	gamete intrafallopian transfer
PROST	pronuclear stage tubal transfer*
IVF-ET	*in vitro* fertilization and embryo transfer
TEST	tubal embryo stage transfer*
Others	
DIPI	direct intraperitoneal insemination
POST	peritoneal ovum and sperm transfer
FREDI	Fallopian replacement of eggs and delayed insemination
ICSI	intracytoplasmic sperm injection

* same as zygote intrafallopian transfer (ZIFT).

sometimes a range of factors appear to be relevant to the case. Indeed, the success of such treatments for poorly explained cases of infertility implies that other factors are operating even when diagnostic investigations reveal discrete abnormalities. A range of gamete manipulation procedures have now been described (Table 3) and these are often or usually combined with ovarian stimulation to achieve optimal results.

Substitution treatments

Substitution treatments provide the only methods currently possible for couples to achieve their own pregnancies when vital reproductive structures or suitable gametes are absent. These include *ovum donation, sperm donation, embryo donation* and *IVF surrogacy*. In the latter case, embryos generated from the gametes of an infertile couple are transferred to a surrogate who carries the pregnancy on behalf of the infertile couple.

Ovarian stimulation

Ovarian stimulation therapy with CC (Fig. 1) and gonadotrophins was introduced into clinical practice in the late 1960s for women with anovulation, usually presenting with amenorrhoea or marked oligomenorrhoea. Normogonadotrophic

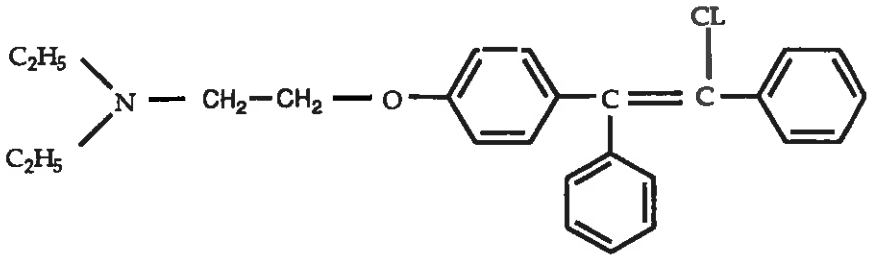

Fig. 1. Chemical structure of clomiphene citrate which has *cis*- and *trans*-isomeric forms, the former having greater biological activity. The molecule is structurally related to diethylstilboestrol.

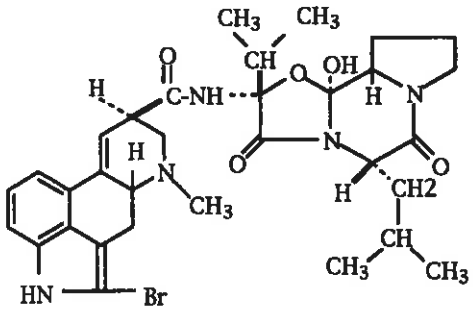

Fig. 2. Chemical structure of bromocryptine mesylate (2-bromo-α-ergocryptine), a peptide ergot alkaloid.

women are usually responsive to CC tablets (50 mg to 200 mg per day for 5 days) revealing signs of ovulation, but pregnancy occurs in only half of responding cases probably due to adverse actions such as cervical mucus inhibition, retention of oocytes within follicles and endometrial receptivity inhibition. Hypergonadotrophic women were found to be unresponsive to any form of stimulation due to ovarian failure and hypo-gonadotrophic cases with hypo-oestrogenism usually required gonadotrophins. These cases have an excellent prognosis with high rates of both ovulation and pregnancy (around 90%). Hyperprolactinaemic amenorrhoea is another anovulatory group with an excellent prognosis, responding well to oral bromocryptine (Fig. 2), usually in dose schedules of 2.5 mg to 10 mg per day. Hyperprolactinaemia requires careful assessment to determine any underlying aetiology such as abnormal thyroid function with thyroid stimulating hormone elevation and consideration of drug-related elevations.

Effective ovulation induction, particularly by bromocryptine and gonadotrophin therapy, generates high rates of pregnancy if other fertility factors for the couple are normal. However, the treatments are not without hazards.

Generally, ovarian stimulation has not been recommended for women who show evidence of ovulation. However, in clinical practice it is apparent that

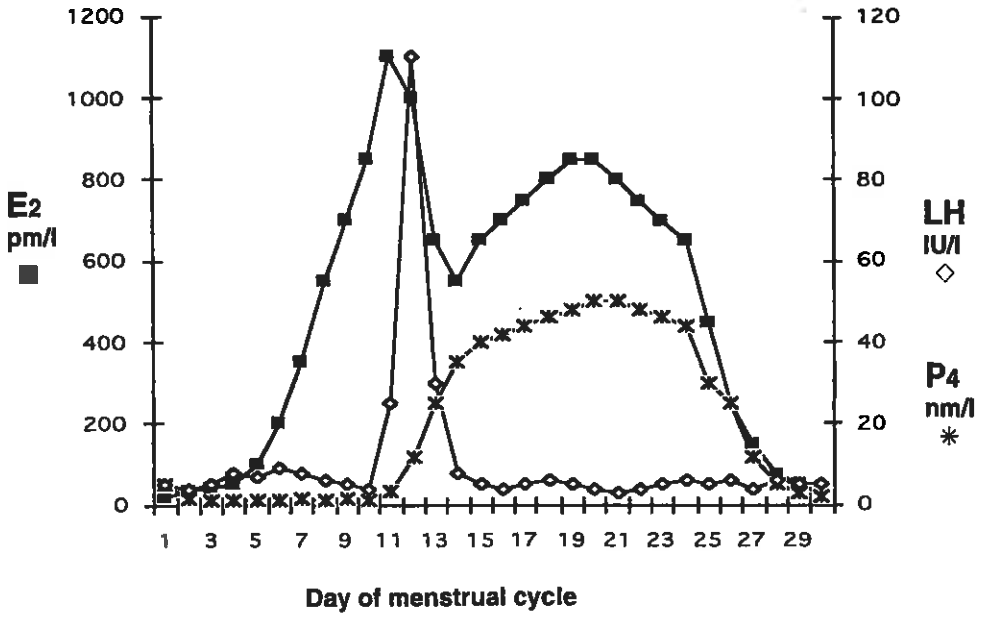

Fig. 3. Hormonal changes during a normal 'conception' ovulatory cycle showing serum oestradiol (E_2), luteinizing hormone (LH), progesterone (P_4) and, if pregnant, the pattern of detectable human chorionic gonadotrophin (βhCG) elevation.

conception can be enhanced by stimulation schedules applied empirically (Yovich *et al.*, 1987) and which probably correct minor disorders of ovulation; as an adjunct to insemination procedures if conception fails to ensue over 4–6 treatment cycles; and as a routine for IVF-related procedures to provide a number of oocytes for fertilization and the subsequent selection of optimal embryos for transfer.

Stimulation for disordered ovarian cycles and empiric stimulation

Women with unexplained infertility who are most likely to respond to ovarian stimulation are those who demonstrate some disorder when their cycles are closely monitored and compared to a normal 'conception' cycle (Fig. 3). Such normal cycles display oestradiol-17β (E_2) rises >620 pm/l prior to LH surge and the pre-surge LH levels throughout the follicular phase should be no greater than 1 standard deviation above the mean for conceiving women (Stanger & Yovich, 1985) (usually <10 i.u./l). The LH surge should be followed by an appropriate rise in progesterone (P_4) and the mid-luteal levels of E_2 and P_4 should be >500 pm/l and 30 nm/l, respectively. Ovarian ultrasound evaluation should exclude polycystic ovaries (PCO) (Adams, Polson & Franks, 1986) and demonstrate a dominant follicle $\geqslant 1.5$ cm at the time of LH surge. Cycle length may also be relevant; in particular the luteal phase dated from LH surge should probably be >11 days. Women with clinical, ultrasound, androgen, or LH

evidence of PCO should be identified and will generally be responsive to ovarian stimulation.

The author's preferred stimulation regimen is sequential as follows:

First, any underlying disorder is corrected where possible (e.g. weight control aiming for BMI between 20 and 25). Thereafter, the response to CC is monitored, beginning with 50 mg/day on days 2–6 of the cycle. The dose can be raised to a maximum of 200 mg/day but it is generally unrewarding to proceed beyond 50 mg b.d. Ideally the cycle should be monitored by BBT changes, hormonal assays, cervical mucus changes and ovarian ultrasound. A triggering injection of hCG 5000 IU can then be given at the appropriate stage of follicle development and booster injections of 1000 i.u. hCG given on days 4, 7, 10 and 13 after the trigger will improve corpus luteal function. Apparently normal ovulation occurs in 70% of cases but pregnancy ensues in less than half, usually within three treatment cycles. The discrepancy is partly due to mucus inhibition which occurs in 22% of CC cycles (Matson & Yovich, 1987), as well as the probable failure of follicles to disperse and release their oocytes properly (Stanger & Yovich, 1984).

If the response to CC has been ineffective, hMG should be added to the regimen. Depending upon the woman's age and weight, this is commenced with 75 to 150 i.u. i.m.i. alternate daily from day 3 of the cycle (i.e. the day after CC begins) increasing by 1 to 2 ampoules after 3 days if the response has been inadequate judged by the daily monitoring of E_2 levels from day 8 or 9 of the cycle. The aim is to generate a steady rise of E_2 over 6 days when the hCG trigger is given, followed by the luteal boosts as described above. One aims for peak E_2 levels between 1000 and 3000 pm/l which matches 1–3 mature follicles. Greater levels and follicle numbers signal a real danger of high-order multiple pregnancy and in such instances the couple should be counselled to consider the alternative options of avoiding intercourse in that cycle or converting to GIFT treatment where the oocyte numbers available for pregnancy can be controlled. The CC/hMG regimen (Fig. 4) is the most effective for empirical stimulation but some cases demonstrate continuing inhibition of cervical mucus (11%), raised LH or raised androgens which appear to be a consequence of the CC. Future cycles should be treated with hMG alone as described in combination with CC. In such cases the luteal hCG boosts are essential to avoid a markedly shortened luteal phase around 9 days.

The standard regimen therefore involves the progression from CC to CC/hMG to hMG alone. Bromocryptine may be used in conjunction to control hyperprolactinaemia and spironolactone to inhibit high androgen effects. However, certain groups of women may still fail to respond adequately to the regimen and are usually identified as those of advanced age, some cases of PCO disease in a partial or impending state of ovarian failure with mildly elevated

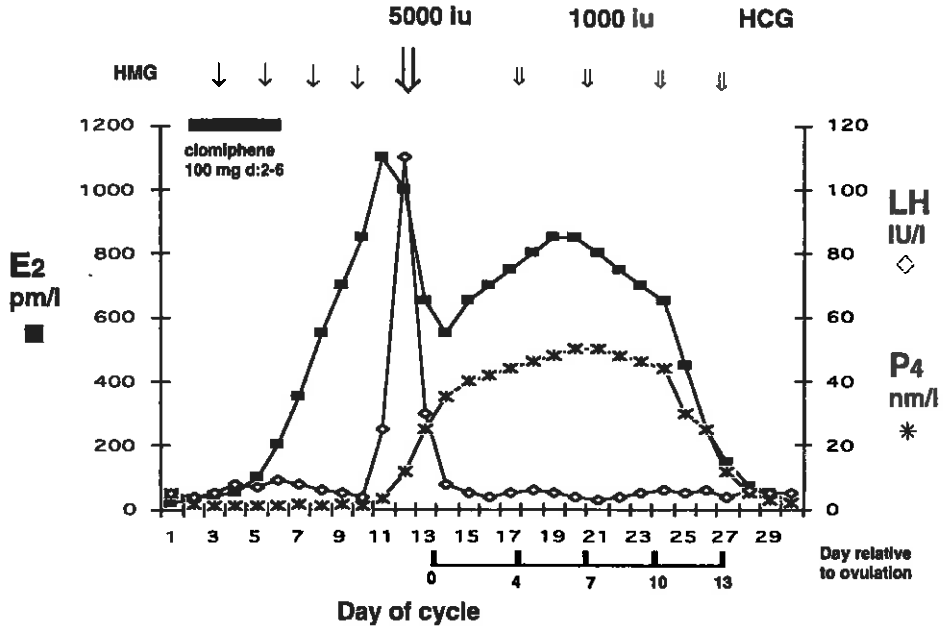

Fig. 4. Ovarian stimulation with clomiphene citrate combined with human menopausal gonadotrophin (CC/hMG) and including human chorionic gonadotrophin (hCG) for ovulation trigger and luteal support.

serum FSH, and others who simply demonstrate ovarian resistance even when 750 IU or more of hMG are given per day. Purified FSH (e.g. Metrodin, Serono) has been evaluated in several trials and may have conferred some benefit for those women with raised basal LH levels. Some cases may benefit from gonadotrophin suppression for 2–3 months using one of the higher strength oral contraceptive preparations. However, a more useful approach has followed the introduction of GnRH analogues which will be described in conjunction with IVF-related treatments.

Ovarian stimulation for insemination treatments

Donor insemination is generally applied as substitution therapy for severe male factor infertility, usually non-correctible azoospermia. The female partner usually has normal fertility or, at least, may not have had the opportunity to test her fertility. Furthermore donor insemination treatment relies on spermatozoal interaction with cervical mucus and the natural processes of sperm migration through the upper genital tract. Therefore ovarian stimulation is best avoided. However, if conception fails to ensue within four to six treatment cycles, ovarian stimulation will improve the chances if investigations of the female fail to detect any abnormalities other than minor disorders of ovulation. In such cases it is

important to evaluate the effect on cervical mucus in each cycle as it is wasteful to undertake intracervical DI in the absence of fertile mucus. In such cases IUI with donor sperm can be applied.

The prognosis for unstimulated DI treatments is 10–15% clinical pregnancies per treatment cycle. IUI treatments with hMG stimulation can generate twice the pregnancy rate but this has to be weighed up against the hazards, particularly a five-fold increase in the rate of multiple pregnancy.

AIH (i.e. IUI with husband sperm) (Yovich & Matson, 1988*b*) poses quite a different set of conditions. First, the nature of the infertility problem is less clear. Even if AIH is indicated for a significant male factor problem, there will undoubtedly be some contribution to the couple's infertility by female-related factors, even if these are not identified from the investigations. Mild and moderate degrees of oligozoospermia are only variably associated with infertility and therefore constitute a relatively significant factor for only certain infertile couples. Furthermore, the sperm preparation technique and the procedure involved for AIH requires freshly released oocyte/s to be present in the fallopian tube at the time of insemination, and there is minimal reliance on the natural process of sperm transport in the female genital tract. For these reasons, ovarian stimulation, or at least ovarian cycle monitoring and usually an hCG trigger, are an integral part of the treatment in order to maximize the chance of viable sperm making contact with the oocyte.

The author's current programme involves the following:
(i) alternate day hMG injections; (ii) hMG dose according to age and previous response injection; (iii) hCG trigger when E_2 and ultrasound observations are optimal; (iv) single IUI at 42 h under ultrasound control; (v) husband's ejaculate collected 90 min prior for preparation.

The above regimen provides clinical pregnancy rates around 20–25% per cycle over three cycles if $\geqslant 5$ million motile sperm are available for insemination and the female genital tract is normal. The sperm may require enhancement accordingly to motility characteristics and acrosome reactivity (Yovich, 1993).

Ovarian stimulation for IVF-related procedures

The pioneer workers in human IVF used hMG stimulation in order to generate several ovarian follicles for oocyte recovery when techniques were relatively crude and to counter the problems of limited fertilization and limited developmental potential of oocytes. However, they recognized that the luteal phase was shortened to a degree which was directly related to the output of urinary oestrogens measured during the follicular phase (Edwards *et al.*, 1980). Subsequently, when a rapid immuno-bioassay (Hi-Gonavis; Mochida Pharmaceutical Co, Japan)

became available for LH/βhCG detection, natural ovulatory cycles were monitored and the single oocyte was 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 hospitalization for 8-hrly 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 the IVF procedure was to be adopted into clinical service.

The first clinic to report success with stimulated cycles (Trounson, Leeton & Wood, 1981) utilized CC alone for stimulation and hCG 5000 IU for the ovulation trigger. Oocytes were recovered 33–35 h later and no luteal support was provided. Subsequently, others, particularly in the United States, reported success using hMG alone for stimulation (Jones *et al.*, 1982), triggering ovulation with hCG 10 000 IU and giving luteal support routinely in the form of progesterone i.m.i. 25–50 mg/day. 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 CC with hMG. Generally 50 mg CC was given b.d. days 2–6 or 5–9 and hMG 75 IU ampoules (1–3) were given beginning a day or two after the CC was commenced. Cancellation rates due to poor or inappropriate responses and premature LH surges were around 20%. Clinics varied in their opinion regarding a 'coasting' phase for hMG prior to the hCG trigger and also regarding the need or value of luteal phase support (see later).

Marked improvements in IVF results have recently ensued from the diminishing use of CC and the increasing use of GnRH analogues such as Buserelin (Suprefact, Hoechst Laboratories) and Leuprolide acetate (Lucrin, Abbott Laboratories). These agents can be used in various regimens combined with hMG. Optimum results appear with a *pituitary down-regulation schedule* (Tan *et al.*, 1992*a*,*b*). A successful regimen is shown in Fig. 5 and involves commencing Lucrin 1 mg s.c. daily in the mid-luteal phase of the preceding cycle. Pituitary down-regulation is usually achieved by day 3–5 of the ensuing cycle and is demonstrated by serum FSH and LH levels <5 IU/l and E_2 <200 pmol/l. Thereafter 0.5 mg Lucrin daily will maintain suppression and hMG injections are given daily with appropriate increases after 3 days of any given dosage in order to increase E_2 by approximately 50% per day and the hCG trigger is given on the 7th day of sustained E_2 rise. Spontaneous LH surges rarely occur with this regimen. Ultrasound monitoring can provide additional useful information and may occasionally lead to delaying the LH trigger until a cohort of follicles have reached 1.6 cms diameter or greater.

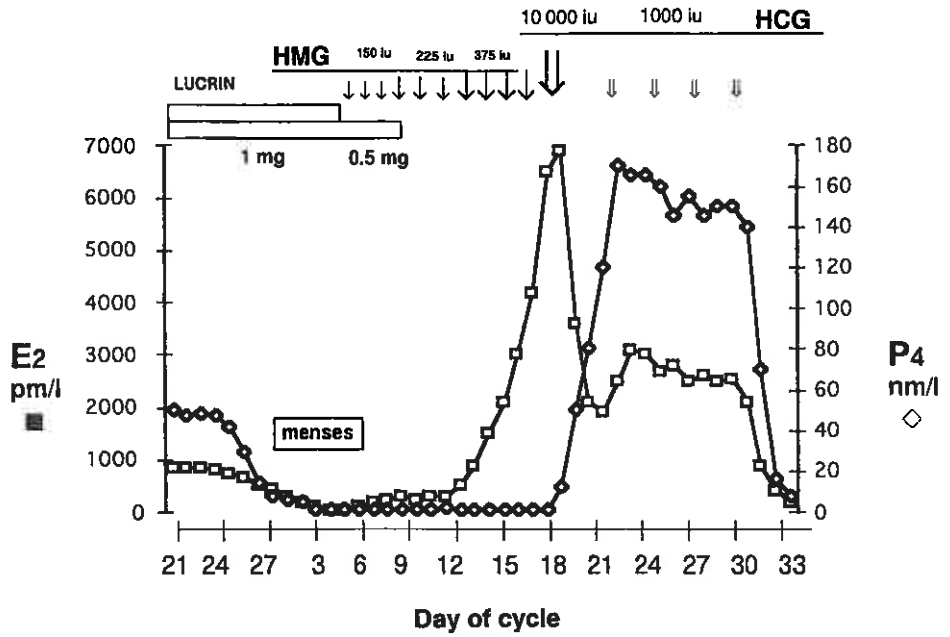

Fig. 5. Ovarian stimulation for *in vitro* fertilization (IVF) using leuprolide acetate (Lucrin) for pituitary down-regulation prior to hMG stimulation and hCG trigger and luteal support. The strength of hMG may be adjusted every 3 days to maintain a rising E_2 level over 7 days.

One aims for E_2 levels around 6000 pmol/l which indicates the likely recovery of 6–8 oocytes. If E_2 levels rise above 10 000 pmol/l there is a significant risk of ovarian hyperstimulation syndrome (OHSS).

The use of GnRH analogues has been shown to have significant benefits in patients of advanced age, with underlying PCO, with raised androgens, with raised basal LH or with previous premature LH surges (Cummins *et al.*, 1990). Those who have previously demonstrated poor ovarian responsiveness may often respond to the *'flare' technique* which involves commencing both the analogue and hMG together at the beginning of the cycle when the analogue will initiate pituitary release of gonadotrophins as a normal effect prior to down-regulation and so supplement the exogenous gonadotrophin. An *ultra-short 'flare' regimen* (Macnamee *et al.*, 1989) has also been described which is proving equally useful and has certain cost benefits although premature LH surges occur more frequently. However, the poor responder group remains a difficult group to treat effectively and cancellation rates due to an inadequate response are of the order of 10%. Current research indicates that the combined use of recombinant growth hormone (rGH) may improve the response or at least reduce the amount of hMG required to effect successful stimulation (Homburg *et al.*, 1990). However, the expense of such treatment is currently prohibitive for consideration in routine clinical service.

Other developments in ovarian stimulation for assisted reproduction include the use of recombinant FSH (Devroey *et al.*, 1993) and the use of GnRH antagonists (Hall, 1993). It remains to be seen if any advantages will be shown.

Timing the hCG trigger injection

Perhaps the main 'art' in assisted reproduction is deciding when to give the hCG trigger injection and upon what criteria to base the decision.

In normal unstimulated cycles, the LH surge occurs around day 12 when fertile mucus can be detected, ultrasound detects a follicle $\geqslant 15$ mm in diameter and he E_2 level is around 1000 pmol/L, usually on day 6 or 7 of the rise from the menstrual baseline (Yovich & Grudzinskas, 1990). In stimulated cycles, cervical mucus may become an unreliable guide as it may be inhibited, e.g. by CC, or enhanced over a prolonged phase, e.g. by hMG so that the chance of pregnancy bears no relationship to cervical mucus score at the time of trigger (Matson & Yovich, 1987). In stimulated cycles clinicians may choose to trigger on the basis of ultrasound findings e.g. leading follicle $\geqslant 18$ mm for IUI or leading follicle $\geqslant 18$ mm and at least 2 other follicles > 14 mm for IVF. They may enhance the decision by E_2 assays, aiming for a ratio of around 1000 pm E_2/L for each preovulatory oocyte. The author's preference is to trigger on day 7 of E_2 rise in GnRH analogue suppressed cycles which is often 2 days later than the aforementioned regimens and which has been shown to lead to a significant improvement in implantation and pregnancy rates (Tan *et al.*, 1992*a*,*b*). The timing may be modulated further by adjusting the trigger awaiting endometrial thickness measured on ultrasound to be at least 8 mm (Gonen *et al.*, 1989).

Luteal phase support

It would appear that regimens incorporating CC usually have normal luteal phase lengths and sustain a satisfactory output of P_4 and E_2. However, this can be ensured and improved, by giving hCG boost injections (Yovich, 1988) (Fig. 4). Although debate continues concerning the role of luteal support therapy, GIFT patients showed significant benefits from luteal support measured by implantation rate, pregnancy rate and livebirth rate, the latter showing the most marked benefit (Yovich, Edirisinghe & Cummins, 1991). Data modelling techniques implied that luteal support was of most value when the embryo quality factor was poorer. Although the author favours the hCG regimen, it was shown that daily progesterone injections i.m. (Proluton 50 mg; Schering UK) were equally effective. However, it appears that continuation of the Proluton injections is required throughout the first 10 to 12 weeks of the pregnancy as the corpus luteum may

become suppressed and fail to respond to hCG secreted from the implanting embryo. The data also showed an apparent further benefit for a combined regimen of hCG and Proluton and early pregnancy support is usually not required. This combined regimen should be considered for cases with repeated failed implantations or poor hormonal profiles. As previously mentioned, cases undergoing hMG stimulation alone including those having either GnRH agonist down regulation or flare methods, have a high rate of luteal phase inadequacy with a very short luteal phase (usually around 9 days) unless luteal support is given. Each of the aforementioned three regimens has been found to be effective.

Hazards of ovarian stimulation

The various drugs used to stimulate folliculogenesis have proven to be relatively free from significant direct side effects, the major problems resulting from the secondary effects of excessive follicle stimulation – namely multiple pregnancies, ectopic and heterotopic pregnancies, OHSS and complications arising in enlarged ovaries.

Indirect hazards

Ovarian hyperstimulation syndrome

OHSS is the main serious and life-threatening complication of ovarian stimulation and is an iatrogenic disorder. A useful classification put forward by the WHO Scientific Group (Lunenfeld, 1976) describes three grades of severity. In its severe form there is massive ovarian enlargement, ascites, pleural effusions, haemoconcentration, oliguria, electrolyte imbalance and hypercoagulability. These changes can potentially lead to severe respiratory embarrassment, renal failure and disseminated intravascular coagulation, all life-threatening conditions. 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 (Golan *et al.*, 1989).

OHSS requiring hospital admission is rare after CC alone but occurs in 1.5%–3% of cycles where HMG is used. Younger women with PCO and highly responsive ovaries are most prone (MacDougall, Tan & Jacobs, 1992). Conversely, those women requiring very high doses of HMG are least prone. Furthermore, the rates may be a little higher in GnRH analogue cycles. Implantation rates and pregnancy rates are significantly higher in cases complicated by OHSS, being around 50% per embryo and >80% per cycle respectively, and the multiple pregnancy rate may also be higher than in comparable stimulation groups. The incidence of OHSS appears to be lower in women undergoing ovarian stimulation

for IVF than for GIFT and this may possibly relate to a beneficial effect of follicle aspiration (Draper & Yovich, 1988).

Treatment includes intravenous hydration, management of any electrolyte imbalance, careful attention to fluid balance and paracentesis with continuous drainage of the ascitic fluid. Such patients should not receive hCG injections during the luteal phase but may continue with progesterone support if luteal therapy is favoured. Abdominal drainage is continued over three to five days and 7 to 12 litres of straw coloured fluid may drain which can be proteinaceous towards the end. Intravenous human serum albumin infusions may also be beneficial. If the patient is not pregnant, the condition resolves spontaneously prior to the menstrual period, otherwise it recedes slowly by the eighth week of pregnancy.

A preventative approach to management involves continuation of the GnRH analogue throughout the luteal phase after oocyte recovery in cases suspected to be of high risk. Embryos are cryopreserved and transferred in the subsequent cycle which may be natural or controlled by a hormone replacement schedule similar to that used for ovum donation. The protocol appears to minimize any tendency to OHSS and the need for paracentesis is uncommon.

Multiple pregnancies

Ovarian stimulation increases the chance of pregnancy significantly for each of the assisted reproduction procedures, but the price is a rise in the rate and numeracy of multiple pregnancies. This is shown by data modelling using the binomial expansion in an IVF programme (Fig. 6). If the individual chance of implantation of a transferred oocyte (in GIFT) or embryo (in IVF) is 15% (a common rate among younger patients in efficient clinics), the pregnancy rate rises to 48% when four oocytes or embryos are transferred, but the multiple pregnancy rate will be 22% and include 1 quadruplet every 1000 pregnancies. The pregnancy rate can be further 'improved' to 56% by transferring five embryos but the multiple pregnancy rate will be 28% and include four quadruplets and possibly one quintuplet every 1000 pregnancies! For this reason, both voluntary and legislative controls place a maximum of three oocytes or embryos transferred, reduced to two in younger women with a favourable prognosis.

Subfertile women have a higher risk of preterm delivery even if a singleton pregnancy is achieved (14% as opposed to 7% in the general obstetric community). The rates are markedly increased for twin and triplet pregnancies with a consequential major rise in the risk of perinatal death or cerebral palsy in the survivors being 7-fold for a twin and 45-fold for a triplet infant when compared with background rates of term infants in the general population (Petterson *et*

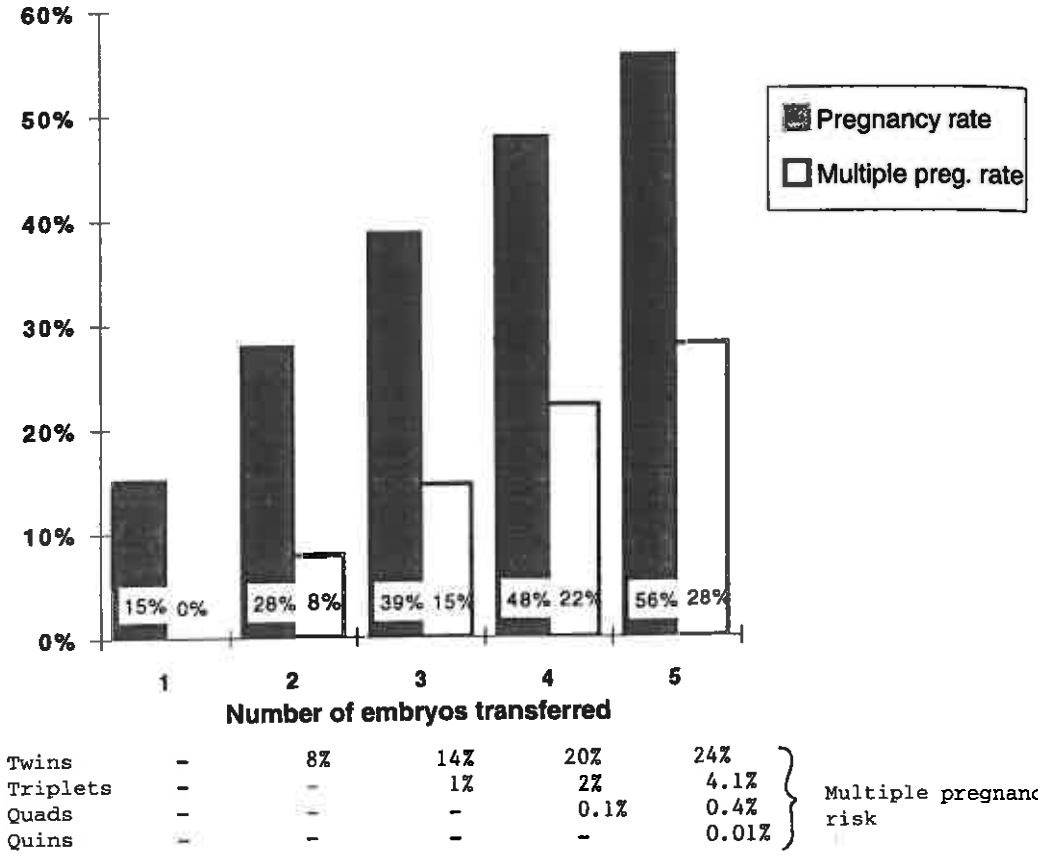

Fig. 6. Pregnancy rates and multiple pregnancy risk from IVF in relation to number of embryos transferred. Data is modelled from binomial expansion, i.e. Rate = $U({}^{n}_{r})E^{r}(1-E)^{n-r}$ using embryo implantation factor (E) of 0.15 and assumes uterine receptivity factor (U) to be 1.0 in conceiving patients.

al., 1993). Again the need for care, control and monitoring of ovarian stimulation is highlighted.

Ectopic and heterotopic pregnancies

Ectopic pregnancies arise in approximately 5% of women treated by assisted reproduction (Yovich & Matson, 1988*a*) and the rate is mainly dependent upon the degree of abnormality of the Fallopian tubes. The rate of ectopic pregnancy in a fertile population is around 1% and this is the rate found in DI treatments where the main basis for the infertility is a well-defined male factor. In GIFT treatments performed in the presence of known tubal disorder, the rate may approach 20%! (AIHW National Perinatal Statistic Unit, 1992) By confining all cases with known tubal disorder to IVF-ET, and transferring the embryos carefully to the mid-uterine cavity in a low fluid volume (Yovich, Turner &

Murphy, 1985), the ectopic rate can be reduced to 2%. However, some cases will still occur for unexplained reasons and occasionally the ectopic will occur in combination with an intra-uterine pregnancy, i.e. heterotopic (Yovich *et al.*, 1984, 1985; Molloy *et al.*, 1990; Svare *et al.*, 1993). The natural incidence of heterotopic pregnancies was calculated as 1 per 30 000 pregnancies but the incidence is now reported as high as 1 per 300 in assisted reproduction.

Complications to enlarged ovaries

These include ovarian torsion, ruptured luteal cysts and pelvic pressure symptoms. Serious sequelae appear to be infrequent.

The question of malignancies

Breast cancer and ovarian cancer have recently been suggested as potential hazards from repetitive ovarian stimulation treatments.

Breast cancer

This is a common malignancy, destined to affect 2% of women by 50 years rising markedly to 10% by 80 years of age. It is therefore inevitable that some women who had ovarian stimulation in the reproductive age will subsequently have breast cancer diagnosed. The risk may actually be higher in subfertile women (Folsom, Sellers & Kaye, 1993) in particular those women with polycystic ovaries who have high unopposed oestrogen levels (Toniolo & Whittemore, 1992), a sizeable group in any infertility clinic, but there appears not to be a direct causal effect from ovarian stimulation therapy. However, long-term prospective matched studies have yet to be reported.

Ovarian cancer

Two separate reports have attracted attention to the possible role of ovarian stimulation drugs – the first detailing epithelial ovarian cancers from an epidemiological study which included women who had a past history of ovarian stimulation (Whittemore, Harris & Itnyre, 1992); the second describing 12 cases of granulosa-cell ovarian cancers in women who had past treatment with CC (Willemsen *et al.*, 1993). However, in both reports the relationship between the treatments and the disease is unclear. Pregnancy and ovulation suppressing contraception both confer a protective effect for ovarian tumours (and also breast and endometrial cancers). Subfertile women may be more prone to ovarian cancer

from incessant ovulation, possibly enhanced by repetitive ovarian stimulation, particularly if pregnancy is not achieved. However, the question of a causal relationship between fertility drugs and ovarian cancer can only be answered by appropriately designed prospective studies (Balasch & Barri, 1993).

Creutzfeld–Jacob disease (CJD)

Human pituitary hormones derived directly from human cadavers (usually young, ostensibly healthy, road accident victims at the time of autopsy) provided a major source of growth hormone and gonadotrophins from the mid-1960s to 1985. Worldwide, the programmes ceased when reports from the United States showed a clustering of CJD, a rare and fatal central nervous system disease which normally occurs in the general population at a rate of one case per million. To the end of June 1993, 45 CJD cases have been documented from 30 000 people treated with either human growth hormone (hGH) or hPG. In Australia, 4 women have died from CJD from only around 1500 treated.

The pituitary gland extracts were prepared in a way which precluded the survival of any known infective agent, either bacterial or viral. However, CJD is thought to be transmitted by a prion, an infectious protein particle which can behave like a virus influencing DNA activity within neural cells and inducing cerebral amyloidoses with spongiform encephalopathy (Brown *et al.*, 1993). The incubation period from presumed inoculation to clinical disease has been around 15 years and, as yet, there is no test to detect who is carrying the infection. hPG programmes were ceased in 1985 and recipients of hPG have been asked not to donate tissues or organs although there is no proof that CJD can be transmitted through blood transfusion.

Direct side effects of ovulation drugs

CC side effects are not prominent and are dose related. Vasomotor symptoms such as hot flushes caused by the anti-oestrogen action and visual symptoms described as scintillating scotomata, disappear on cessation of the drug. However, the author is aware of one case with some permanent partial visual loss. Whether this was a direct effect of CC or a secondary effect of raised E_2 is uncertain. Other oral stimulants include cyclofenil and tamoxifen and there may be some differences among them with respect to cervical mucus inhibition and effects on endometrial morphology (Suginami *et al.*, 1993; Asaad *et al.*, 1993; Thompson *et al.*, 1993).

Bromocryptine commonly causes nausea, dizziness and headaches during initiation of therapy and, less frequently, nasal congestion, a sense of fatigue and

postural hypotension. These symptoms are usually not severe, and recede if the drug is introduced gradually and tolerance allowed to occur. Occasional serious side effects include seizures and depression.

Menopausal gonadotrophins, hCG and rGH appear to have no significant direct side effects although overdosage of the latter may cause disturbed glucose metabolism and, in long term overdosage, symptoms of acromegaly. GnRH agonists and antagonists also appear to have no significant direct side effects but a range of adverse reactions may arise from their known physiological function e.g. calcium depletion from bones in long-term use associated with prolonged hypo-oestrogenism.

Oocyte recovery techniques

Historically oocyte recovery developed as a laparoscopic procedure but has increasingly become replaced by ultrasound-directed techniques, particularly the trans-vaginal approach. The optimization of oocyte recovery has been shown to depend upon three main aspects (Yovich, Matson & Yovich, 1989):

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;
3. Accessibility of the ovaries for aspiration.

With respect to timing, the LH surge or hCG trigger should occur on day 6 or 7 of CC/HMG cycles and the optimal trigger is day 7 of cycles down-regulated with GnRH analogues (see earlier discussion). Thereafter, follicles are aspirated at 36 ± 2 h after initiation of the LH surge or hCG trigger. Oocytes aspirated earlier than 34 h 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 h earlier than optimal. Oocytes collected up to 4 h after the optimal time remain equally suitable but the risk of spontaneous oocyte release increases. However this risk appears to be $<10\%$ up to 42 h in GnRH agonist 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 have been

recommended. Whilst this has significantly improved laparoscopic access, it may prejudice trans-vaginal access 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 provide optimal oocyte recovery rates, being around 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 the adjacent IVF laboratory, the follicle is flushed with HEPES-buffered flushing medium (HTFM: human tubal fluid medium) up to 2 occasions (total 10 ml) prior to moving to the next follicle.

The post-operative 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 (Lenz, Lauritsen & Kjellow, 1981) and described a transcutaneous transvesical method. Subsequently a transurethral method was explored briefly and finally the transvaginal method has found popular acceptance (Wikland *et al.*, 1989). The optimization of transvaginal ultrasound-directed aspiration requires the following (Yovich & Grudzinskas, 1990):

1. Minimal anaesthesia, e.g. i.v. sedation with medazolam and fentanyl; propofol intravenous anaesthetic; or premedication combined with local anaesthesia.
2. Apply pressure band to lower abdomen to stabilize the ovaries and prevent them slipping away during attempts at needle penetration.
3. Use of very sharp, disposable needles with echo-enhanced tips and which enable an efficient follicle flushing technique. The 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 that 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 mm Hg whilst 25 cm needle requires 100 mm Hg) and needle diameter (inversely related to fourth power of the radius).
6. High resolution ultrasound image required, e.g. General Electric electronic phased array sector scanner with 5.0 MHz vaginal probe and needle guide is widely and effectively used.
7. Ideally the vaginal probe is not covered although a non-toxic condom or clear plastic wrap can be used. The coupling medium is 10 ml culture medium placed in the vagina at the beginning of the procedure after saline washout. Sterilizing fluids are avoided hence it is imperative to exclude the presence of vaginal pathogens just before the treatment cycle. The probe can be sterilized in glutaraldehyde but must be washed thoroughly with sterile water and aired prior to use. Small traces of glutaraldehyde on the probe or within the theatre atmosphere are highly embryotoxic.

Using the above system, the overall oocyte recovery rate is 88% of follicles entered (Hussein, Balen & Tan, 1992). Of interest the vast majority of oocytes were recovered from the follicular aspirate of the first 2–3 ml flush and it was these which were most likely to generate pregnancies.

Outcome of assisted reproduction pregnancies

Early pregnancy wastage after IVF-related treatments appears to be increased above the normal expected rate, but is probably not increased above a matched group of subfertile patients who conceive spontaneously (Yovich & Matson, 1988*a*). As discussed previously, the ectopic pregnancy rate is high, around 5%, and the rate is influenced by both operator and patient factors. Heterotopic pregnancies are also increased. Blighted ovum pregnancies are more common following AIH and where GIFT has been applied for male-factor cases (Yovich, 1993). Preclinical pregnancies may be diagnosed in up to 10% but this rate may not be higher than found in normally fertile women monitored through the menstrual cycle into pregnancy (Wilcox *et al.*, 1988). Late pregnancy outcomes of IVF-related pregnancies reveal a high morbidity and mortality due to an increased risk of preterm delivery, mostly as a consequence of multiple pregnancies (Lancaster *et al.*, 1985). However, even singletons deliver preterm twice as commonly as women of normal fertility and this finding appears to be similar for subfertile women conceiving without assisted reproduction. The overall rate of major congenital abnormalities (IVF: 2.2%; GIFT: 3.1%) does not appear to be increased and the collaborative Australian and New Zealand data which earlier

showed higher than expected observations of infants with spina bifida and transposition of the great arteries following IVF and of infants with major urinary tract malformations following GIFT is no longer sustained (AIHW National Perinatal Statistics Unit, 1992).

Ethical and legal status

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 (Knoppers & Le Bris, 1993). There are four broad areas of concern:

(i) standards of laboratory and clinical practice;
(ii) accountability to the general community;
(iii) protecting the welfare of children born following assisted reproduction; and
(iv) 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. Current topical debates concern access to identifying information and the question of respective responsibilities with regard 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 in assisted 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 industrialized 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, e.g. 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 (Braude & Johnson, 1989). Such legislation may effectively seal the current technology in its relatively inefficient state.

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