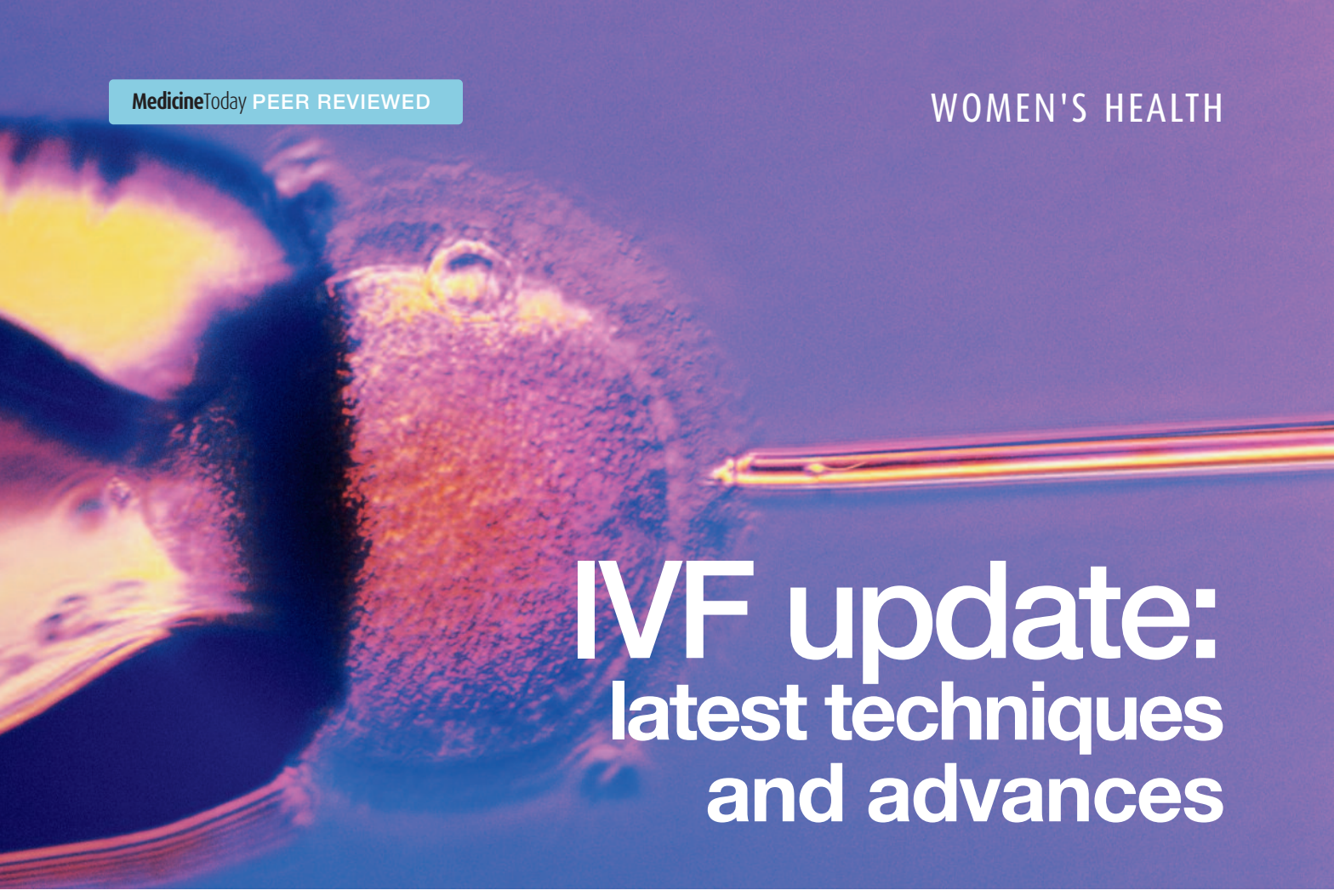

IVF update: latest techniques and advances

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The techniques used in *in vitro* fertilisation continue to evolve as we strive to improve success rates while minimising multiple pregnancy. All advances in techniques should undergo adequate scientific scrutiny and unproven therapies be reserved for appropriate clinical trials.

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The basic concept of *in vitro* fertilisation (IVF), involving ovarian stimulation, sperm collection, fertilisation and embryo culture in the laboratory, followed by embryo replacement into the uterus, has remained largely unchanged since its introduction in the 1970s. There have been dramatic refinements in the technique, however, which have contributed to the enormous advances in success rates over the past three decades.

This article aims to highlight the latest techniques and advances that have contributed to the improvement of IVF, beginning at ovarian stimulation and sperm retrieval, moving on to the uterine environment and advances in the laboratory, and finishing with a brief discussion of oocyte cryopreservation, preimplantation screening and reproductive tourism.

Patients embarking on the IVF procedure are often presented with a myriad of choices and there remains much uncertainty regarding the 'best' treatment for any particular patient.

CONTROLLED OVARIAN HYPERSTIMULATION

During the IVF procedure, the ovaries are stimulated to allow collection of sufficient quantities of mature oocytes. Sufficient quantities are needed to overcome the inefficiencies that are inherent both in the process itself and in the biology. The use of gonadotropins for ovarian stimulation, gonadotropin-releasing hormone (GnRH) analogues to control ovarian response and prevent premature luteinising hormone

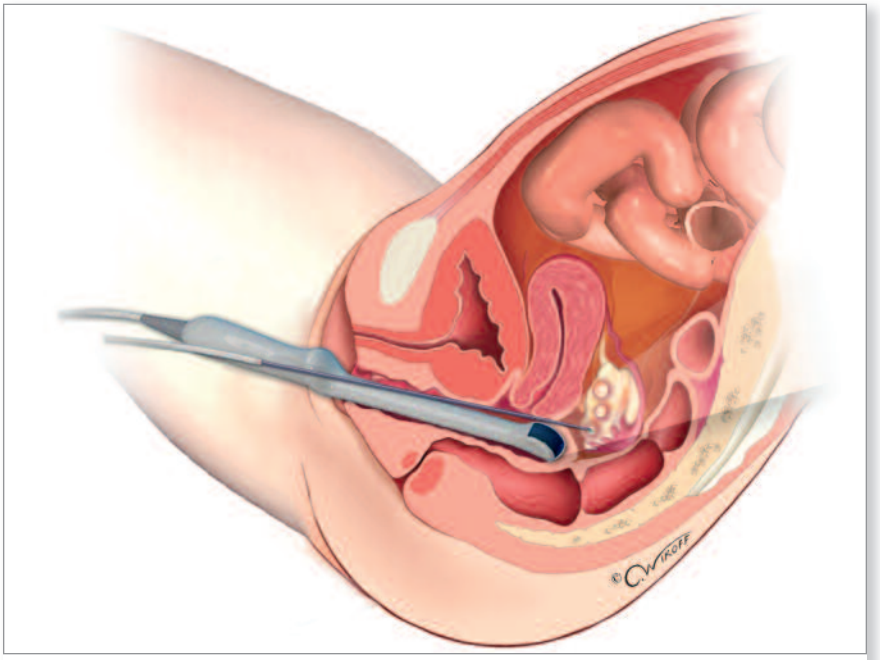

Figure 1. Ultrasound-guided egg collection.

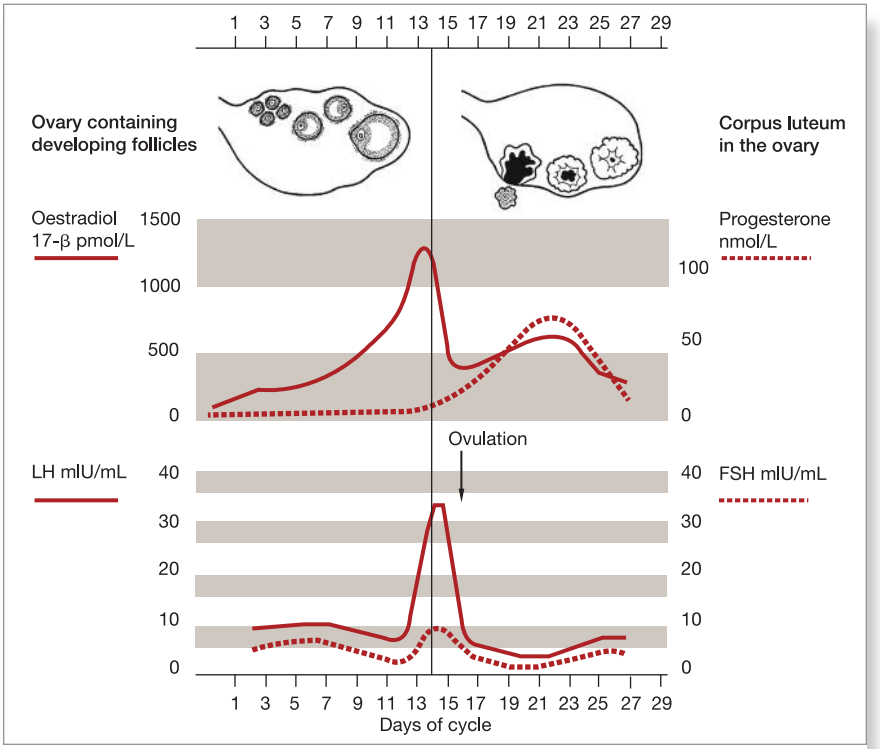

Figure 2. Schematic diagram showing the menstrual cycle.
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ABBREVIATIONS: FSH = follicle-stimulating hormone; LH= luteinising hormone.

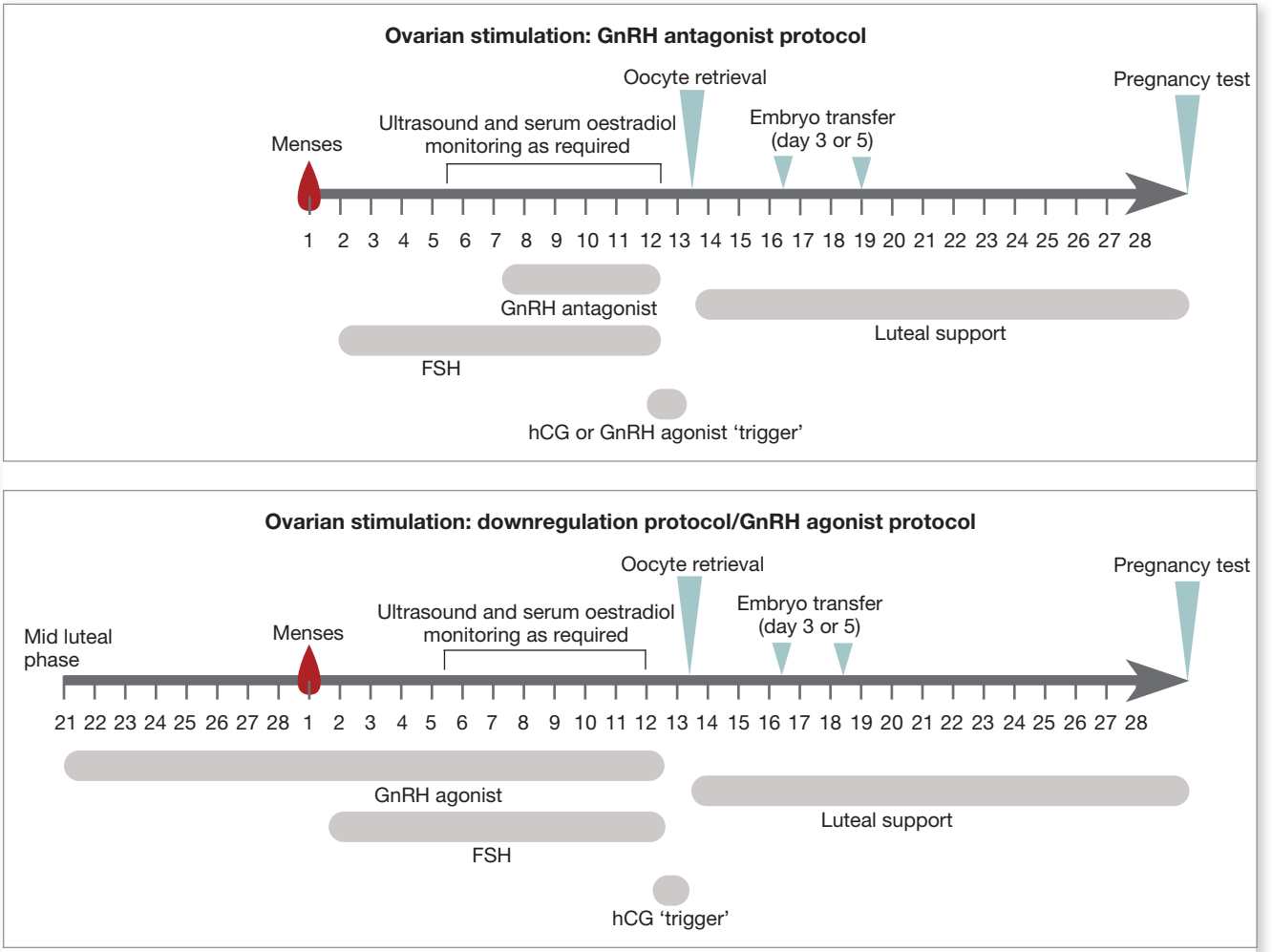
(LH) surge, and transvaginal ultrasound-guided oocyte retrieval (Figure 1) significantly improve the oocyte yield and allow procedures to be scheduled during daylight hours.

Stimulation of follicular development is achieved by daily injection of gonadotropins. The original gonadotropins were derived from the urine of menopausal women; however, most centres now use recombinant follicle-stimulating hormone (FSH). Most women do not require the addition of LH for a successful outcome, although it is essential in women with hypogonadotropic hypogonadism and is currently being popularised for various other indications, for which the evidence is eagerly awaited.[1]

GnRH analogues may be agonists or antagonists. Agonists bind to and stimulate the GnRH receptors but ultimately cause internalisation and depletion of receptors (downregulation) and hence suppression of the LH surge. In contrast, antagonists block the GnRH receptor without any stimulatory effect. GnRH antagonists have become the most popular GnRH analogue in many centres. The major advantage of the antagonist over agonist protocol is that it shortens the treatment cycle for the patient.

An agonist should generally be started in the luteal phase of the menstrual cycle before FSH stimulation to downregulate GnRH receptors and to avoid the 'flare' effect, whereas an antagonist need only be started on approximately day six of FSH stimulation (see Figures 2 and 3). This allows women to start an IVF cycle with their next menstrual period and avoids oestrogen deficiency symptoms that are sometimes experienced with the traditional downregulation agonist cycle. Antagonists are also associated with a decreased risk of ovarian hyperstimulation syndrome and decrease the total FSH requirements.

Although the literature now suggests that the agonist and antagonist protocols are statistically equivalent in terms of



Figures 3a and b. Ovarian stimulation. a (top). GnRH antagonist protocol. b (bottom). Downregulation protocol or GnRH agonist protocol.

ABBREVIATIONS: FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; hCG = human chorionic gonadotropin.

live birth rates, there appears to be some situations where the more traditional downregulation approach has an advantage. These situations include women with significant endometriosis or who have had poor embryology in a previous antagonist cycle.[2]

Within an antagonist cycle, GnRH agonists may be used as an alternative to human chorionic gonadotropin (hCG) for the trigger of ovulation. This practice is associated with a decreased risk of ovarian hyperstimulation syndrome and an improvement in proportion of mature oocytes collected; however, the optimum luteal phase support following an agonist trigger has not been determined, and fresh pregnancy rates are currently lower than with a conventional hCG trigger.[3]

Luteal phase supplementation can take the form of vaginal progesterone pessaries or gel, intramuscular progesterone, hCG injections or a combination of these, with or without the addition of oestrogen. Current evidence suggests that two weeks of use of vaginal pro - gesterone is adequate support; however, the evidence is of poor quality and many different regimens are used in current practice.[4]

Mild stimulation is the current 'catch phrase' in the IVF world. There is now good evidence that live birth rates do not improve and in fact decrease once oocyte yield per egg collection gets above 12 eggs. Australasian clinics have had a much more conservative approach to ovarian stimulation than those in Europe and the USA for some time, so mild stimulation is not that new in Australia. Use of an ovarian reserve marker, anti-mullerian hormone, allows for better decision making regarding the dose of FSH to be administered.[5,6]

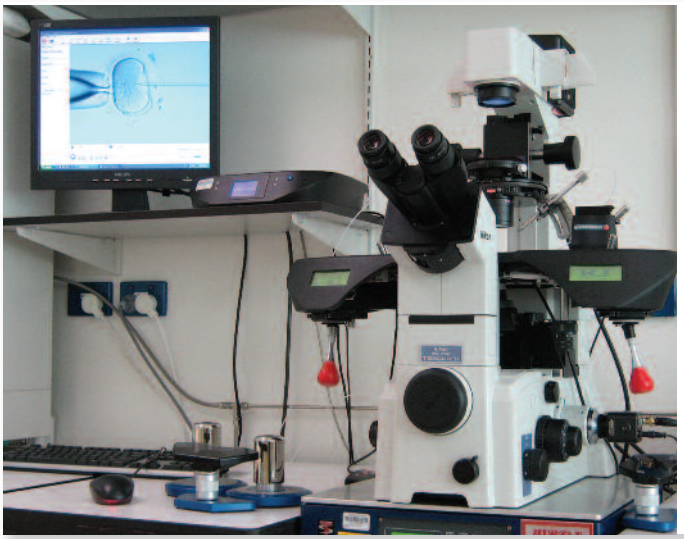


Figure 4. ICSI microscope setup for sperm microinjection.

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Injection of corifollitropin alfa, a long-acting FSH, is an exciting development that may decrease the woman's treatment burden. One injection of long-acting FSH is generally adequate for seven days of stimulation and generally only one to two further FSH injections are needed before egg collection. This preparation only became PBS listed in December 2011. As experience with this preparation increases, it is likely that indications for its use will become more widespread.[7]

Women who are poor responders present a vexing problem to fertility clinicians. The use of additional supplements such as dehydroepiandrosterone, testosterone patches or growth hormone to conventional stimulation regimens is common in many centres, and often encouraged by patients who have been reading widely on the Internet. Unfortunately, there is currently no good evidence that any of these measures improve outcome and it is important that patients are informed of this.[8] Growth hormone is probably the medication with most potential and a large Australasian randomised controlled trial is currently underway in an attempt to prove this.[9] Outside of the trial, growth hormone is an unproven and very expensive treatment.

SPERM

Intracytoplasmic sperm injection

The introduction of intracytoplasmic sperm injection (ICSI) in 1990 revolutionised treatment of the infertile male (Figure 4). For conventional IVF, about 50,000 progressively motile sperm after sperm preparation are required to achieve successful fertilisation. Using ICSI, a single viable sperm is injected into each oocyte to achieve average fertilisation rates of about 65%. For men with azoospermia, sperm can often be retrieved from the testes to be used for ICSI.

ICSI is associated with a small increase in birth defects compared with spontaneous conception and IVF. A recent Australian study has shown the unadjusted risk of birth defects were 5.8%, 7.2% and 9.9% for spontaneous conception, IVF and ICSI, respectively.[10] The adjusted odds ratio for ICSI was 1.57 (95% confidence interval, 1.30 to 1.90).[10] It is possible that this increase reflects the increase in chromosomal abnormalities associated with severe sperm abnormalities; however, an association with the technique itself has not been excluded. This recent confirmation reinforces the opinion that ICSI should be reserved for those who absolutely require it and not be used unnecessarily.

Microdissection testicular sperm extraction

Microdissection testicular sperm extraction is a relatively new technique that can improve the chance of retrieving sperm from a man with nonobstructive azoospermia. This technique involves using an operating microscope to identify microscopic foci of spermatogenesis within the testicular parenchyma rather than using the traditional random biopsy approach. Sperm retrieval rates of up to 63% have been reported in some series with this new technique compared with about 45% with conventional open or needle biopsy.[11]

It has also been reported that sperm retrieval using microdissection testicular sperm extraction was successful in up to 69% of men with nonmosaic Klinefelter's syndrome and in 32.4% of men with sertoli cell only on initial testicular biopsy.[12,13] Despite a more prolonged operating time and extensive dissection, reported short- and long-term complication rates are lower compared with conventional techniques. However, it is still recommended that these men have regular follow up of their hormonal profile to detect subsequent hypogonadism.[12,14]

THE UTERUS

The uterus has gained little attention until recently despite its crucial role in pregnancy. Recent studies of the endometrium have highlighted that the environment resultant from a stimulated IVF cycle is considerably different from that of a natural menstrual cycle and it is postulated that this may be responsible for some implantation failure and obstetric complications.[15-17] Unfortunately, investigation of the human endometrium within conception cycles is extremely difficult due to the likely disruption of implantation and pregnancy.[18]

Endometrial biopsy for assessment of immunological cells, including natural killer cells, is popular in some centres. To date there are conflicting data on

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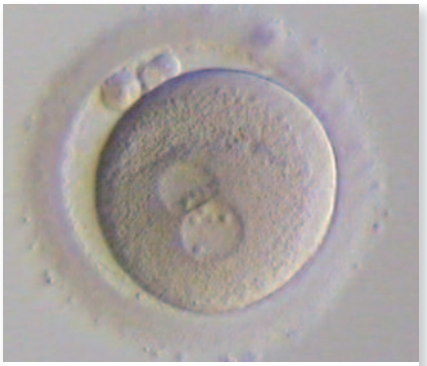

Figure 5. Normally fertilised embryo on day one (two pronuclei).

Figure 6. Embryo at day three (eight cell).

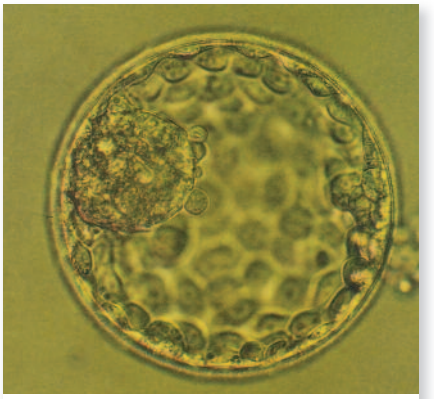

Figure 7. Embryo at day five (blastocyst).

the role of uterine natural killer cells on implantation failure and recurrent miscarriage, largely because no control data are available.[13] Despite this, various different immunomodulating treatments are offered. So far, clinical studies have shown that implantation rates are not improved by the use of systemic corticosteroids, intravenous immunoglobulins or lymphocyte immune therapies.[18,19]

IN THE LABORATORY

The excellent live birth rates currently expected of IVF clinics would not be possible without the major advances in the embryology laboratory.

Blastocyst culture

Extended embryo culture or 'blastocyst culture' involves culturing embryos in the laboratory until day five or six post-fertilisation rather than the traditional day two or three (see Figures 5 to 7). The success of blastocyst culture has occurred due to the recognition of vastly different nutrient requirements compared with the cleavage stage embryo but is highly reliant on meticulous quality control within the laboratory. Without excellent laboratory techniques, any advantage of extended culture is lost due to poor embryo development.

The major advantage of blastocyst culture is that it generally allows better selection of the best quality embryo to replace based on morphological assessment of the embryo, thus improving the implantation rate per embryo transferred.[20] One disadvantage of blastocyst culture is the increased rate of monozygotic twinning of 2 to 4% compared with that of cleavage stage embryos, which is 0.5%.[21]

Growth factors

The addition of growth factors to IVF culture media is one of the latest and controversial advances in *in vitro* culture systems. *In vitro* culture conditions are generally considered suboptimal and deprive the developing embryos of their natural growth factor-rich environment. Recently, the addition of granulocyte-macrophage colony-stimulating factor, a cytokine growth factor involved in growth and differentiation of the trophoblast, has been shown to significantly improve implantation and ongoing pregnancy rate in women with a history of pregnancy loss (unpublished data). This exciting development paves the way for further advances in culture media.

Advanced sperm selection techniques for use in IVF or ICSI are of growing interest because the current standard methods of sperm preparation, such as density gradient centrifugation and swim-up techniques, select only towards motile morphologically normal sperm and cannot select out other characteristics such as DNA integrity, membrane maturation, apoptosis and ultrastructure. These advanced methods include selection based on sperm surface charge, sperm membrane maturity, sperm ultramorphology (motile sperm organelle morphology examination and intracytoplasmic morphologically-selected sperm injection) and nonapoptotic sperm selection. Further studies are awaited to establish the safety and position of each of these methods in clinical practice.[22]

Cryopreservation

Cryopreservation of excess embryos is essential to the 'per stimulated cycle' success of IVF. Vitrification is a technique that has become widespread throughout IVF laboratories. It combines use of concentrated cryoprotectant solutions with rapid cooling, allowing samples to reach low temperatures in a glassy state that has the molecular structure of a viscous liquid rather than crystalline, hence avoiding ice formation. Compared with conventional 'slow freezing' methods, vitrification has been associated with improved cryosurvival of embryos (>90%), particularly blastocysts, higher implantation rates, fewer miscarriages and higher live birth rates.[23] There is increasing evidence that pregnancy rates and obstetric outcomes following transfer of single thawed cryopreserved blastocysts are better than transfer of a fresh embryo in a stimulated cycle (perhaps because of

GP'S COMMENTS

In vitro fertilisation technologies are increasingly employed as the fertility rates of couples are falling in the west as women postpone pregnancy into their late 30s and early 40s. Female infertility rises steeply after the age of 35 years and by the age of 40 years only about 40% of women are able to conceive by natural intercourse. The likelihood of becoming pregnant falls from approximately 85% at the age of 20 years to approximately 5% at 45 years of age. Conversely the likelihood of infertility rises steeply after 39 years of age from 32% to 100% at the age of 50 years. Male fertility also declines with increasing age but occurs at a slower rate than in females. The combination of older males and females attempting conception increases the risks for infertility and the consequent need for assisted reproductive technology.

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adverse effects of stimulation on the endometrium) and it may be that this becomes the standard of care in the future.[24]

With significant improvements in embryo quality from appropriate ovarian stimulation and excellent embryology laboratory techniques, including cryopreservation of embryos, elective single embryo transfer has become the accepted standard.[25,26]

OOCYTE FREEZING

It is now technically possible (and feasible) to freeze oocytes obtained following a standard IVF stimulation protocol before fertilisation. The uptake of oocyte cryopreservation is increasing with indications including: fertility preservation of patients with cancer or for social reasons; ovum donor programs; oocyte accumulation in low-responder patients; and surplus oocyte storage if embryo cryopreservation is not acceptable. The introduction of vitrification over slow freezing has greatly improved oocyte survival, fertilisation and rate of development of top- quality embryos. In some studies, the rate of ongoing pregnancy does not differ between vitrified and fresh oocytes.[27]

PREIMPLANTATION GENETIC SCREENING

Preimplantation genetic diagnosis (PGD) has been available since 1989. This has mostly involved taking a biopsy of one to two cells from a cleavage stage embryo and using the molecular techniques of polymerase chain reaction or fluorescent *in situ* hybridisation to achieve the diagnosis for a single gene disorder or inherited chromosomal abnormality or for sexing for X-linked disease. Normal embryos are then typically transferred into the uterus as blastocysts (day five of development). Preimplantation genetic screening (PGS) uses PGD technology to screen embryos for aneuploidy to select chromosomally normal embryos, as an additional means of embryo selection, for transfer within an IVF cycle.

Although there are several theoretical advantages to PGS, unfortunately there are currently no published randomised controlled trials that show an increase in live birth rate and in fact several show a significant decrease in delivery rates.[28-30] It is possible that newer techniques such as blastocyst biopsy and array comparative genomic hybridisation may overcome several of the technical issues thought to limit the effectiveness of PGS. Until appropriate prospective randomised controlled trials demonstrate a benefit to patients, the use of PGS should be considered experimental.[31]

REPRODUCTIVE TOURISM

It would appear that seeking reproductive treatment across borders is becoming increasingly common, although it is difficult to get an accurate estimate of incidence. The reasons patients choose to travel for reproductive treatment are varied but may include economic, regulatory, legal or ethical restrictions on treatment, a real or perceived shortage of donor gametes, a search for specific expertise or a desire for privacy.

The ethical and legal ramifications for local clinics assisting patients in cross-border treatment can be complicated, particularly when gametes are being obtained on a commercial rather than altruistic basis. Therefore they must exercise caution in the extent of their involvement. Cross-border surrogacy is of particular concern because the child born from such an arrangement may be legally stateless and not be allowed to return with its genetic parents.[32,33]

CONCLUSION

The techniques used in IVF continue to evolve as we strive to improve success rates while minimising multiple pregnancy. Clinicians are often under pressure to modify treatment regimens in the face of unsuccessful outcomes. However, we should all remain cognisant of the fact that currently live birth rates generally do not exceed 50% per stimulated IVF cycle, even in very young women with an excellent prognosis; in older women or those with comorbidities rates are dramatically lower. It is important that all advances in techniques undergo adequate scientific scrutiny and unproven therapies be reserved for appropriate clinical trials. MT

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IVF update latest techniques and advances

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