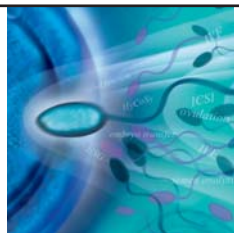

The ART of assisted reproductive technology

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BACKGROUND One in six Australian couples of reproductive age experience difficulties in conceiving a child. Once a couple has been appropriately assessed, assisted reproductive technology (ART) techniques can be used to overcome problems with ovulation, tubal patency, male fertility or unexplained infertility.

OBJECTIVE This article discusses the range of ART techniques available to subfertile couples.

DISCUSSION Intracytoplasmic sperm injection is now the most common form of ART used in Australia, being used in almost half of all fresh cycles, with in vitro fertilisation being used in approximately one-third of all fresh cycles.

The Australian Institute of Health and Welfare's National Perinatal Statistical Unit's annual report *Assisted reproductive technology in Australia and New Zealand 2002*, shows that babies born in 2002 using assisted reproductive technology (ART) had longer gestational ages, higher birth weights and fewer perinatal deaths than their counterparts of 2 years previously.[1]

This is the first year national data have been available on the age of women (and their partners) using ART. The average age of women undergoing treatment in 2002 was 35.2 years, and the average age of partners was 37.6 years.[1] Age remains the most significant issue affecting a couple's chance of conceiving and carrying a pregnancy to full term. Women need to understand the impact age has on their chance of becoming pregnant. Once a couple has been assessed and investigations suggest subfertility, the treatment options depend on the underlying cause (*Table 1*).

Of the ART treatments now in use, intracytoplasmic sperm injection (ICSI) is the most common, being used in 48.4% of all fresh cycles. It now surpasses in vitro fertilisation (IVF) (37% of all fresh cycles), with gamete intrafallopian transfer (GIFT) dropping from 36% in 1993 to just 1% in 2002.[1]

Intrauterine insemination and ovulation induction are commonly used to treat infertility in women who have normal, healthy fallopian tubes and where more complex treatments are not appropriate.

Ovulation induction

In treating unexplained infertility where ovulation is definitely occurring, ovulation induction (OI) with either clomiphene citrate or gonadotrophin, is a typical first step. The aim is to form multiple follicles with eggs for release.

Clomiphene citrate is an oral medication that competes with endogenous oestrogen for oestrogen binding sites on the hypothalamus, and thus blocks the negative feedback of endogenous oestrogen. Gonadotropin releasing hormone (GnRH) is then released in a pulsatile manner, stimulating luteinising hormone (LH) and follicle stimulating hormone (FSH) release. It is usually given for 5 days, starting early in the menstrual cycle.

In our experience, a 20–22% pregnancy rate can be achieved with an 11% chance of conceiving twins without having to proceed to more involved and expensive IVF treatment. Clomiphene citrate is usually prescribed to women who are anovulatory. It can also be used in women who are ovulating in an effort to increase the number of eggs ovulated and thereby slightly improve the chances of conception. Clomiphene citrate, however, does have some potential negative effects including thick cervical mucus, possibly a thin endometrium, and multiple pregnancy.

In our experience, approximately 80% of women will ovulate using clomiphene citrate and half of these will conceive (ie. 40%) within 6 months of treatment beginning. Side effects with clomiphene citrate are common and include hot flushes, occasional mood changes or depression, abdominal bloating, and visual disturbances. Most are mild and disappear following discontinuation of therapy.

Intrauterine insemination

Intrauterine insemination (IUI) is the placing of concentrated sperm into the uterine cavity, bypassing the cervix. This is performed close to the time of ovulation. Intrauterine insemination may be suggested for couples with unexplained subfertility, tubal disease where one tube is normal, mild endometriosis, and mild male factor subfertility. Intrauterine insemination can be used in the following ways:

- in a natural cycle – this is not a good method for women whose partner has poor sperm quality
- with clomiphene citrate to obtain a pregnancy rate of 10–15% per cycle
- with FSH this is the most successful IUI method to create multiple follicular development and thereby improve the chances of conception (15–20% per cycle for women <38 years of age). However, close monitoring is essential to control the potential for multiple pregnancy and ovarian hyperstimulation. The success rate for women aged 40 years and

Table 1. Treatment options for subfertile couples

Cause	Investigations	Treatment options
Ovulation failure	Hormone assessment	Fertility drugs (tablets or injections) Rarely ovum donation
Blocked or damaged fallopian tubes	Laparoscopy Hysterosalpingogram (HSG)	Tubal microsurgery, IVF
Endometriosis	Laparoscopy	Surgical/laser treatment, drug therapy, IVF
Fibroids	Laparoscopy, hysteroscopy, ultrasound scan	May not need treatment but if necessary, can be removed surgically, often laparoscopically
Hostile cervical mucus	Postcoital test (PCT), confirm ovulation, antisperm antibody test	Intrauterine insemination (IUI), IVF
Failure of sperm production	Initial semen analysis ('sperm count'), hormone assessment, testicular biopsy	Surgical sperm collection (SSC), donor sperm
Blocked/absent vas deferens	Scrotal examination, screen for cystic fibrosis	Unblock microsurgically, SSC with IVF/ICSI
Low sperm numbers and/or poor sperm movement	Semen analysis	IUI, IVF or ICSI
High numbers of abnormal forms	Semen analysis	IVF or ICSI
Antisperm antibodies	Antisperm antibody screen	Steroids rarely used, sperm preparation for IUI, IVF or ICSI

over unfortunately is very low, and consequently not frequently recommended.

Blood tests measure hormone levels and ultrasound measures ovarian follicle size and number.

In vitro fertilisation

Mature eggs are collected from the ovary after multiple follicle ripening and placed with sperm in vitro (in 'culture medium' in the laboratory) (*Figure 1*). One or two of the resulting embryos are transferred into the woman's uterus 2–5 days later. The extra embryos are usually stored in liquid nitrogen for later use.

Fertility specialists are now focussed on reducing the number of multiple pregnancies previously linked to ART, and consequently the associated health risks for both mother and baby. The success rate per embryo transferred resulting in pregnancy and live birth has improved dramatically due to critical improvements in the laboratory and clinical management of patients. Advancements in the laboratory include improved culture medium, which more closely mimics the natural environment of a woman's body, and individual incubators that control the environment around the eggs, sperm – and most importantly – the embryos.

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection is a more sophisticated method of achieving fertilisation in vitro and is now the most common method of ART.[1] A single sperm is 'picked up' and injected directly into an egg to assist fertilisation (*Figure 2*). This method is very successful in overcoming problems of male infertility (eg. very few sperm, low mobility, abnormal shape or sperm absence).

Birefringence visualisation of the chromosomal spindle position of eggs during ICSI is a new technique that shows promise in improving embryo development and may be suggested when previous embryo development has been poor.

Cryopreservation

Couples undergoing IVF treatment may choose to have 'extra' embryos frozen for future transfer. This avoids additional treatment cycles and repetitive egg retrieval processes. Embryos can be frozen from the four cell stage to the blastocyst stage. In the 20 years experience in embryo freezing, there have not been any problems with cryopreservation or pregnancy rates.[2]

Blastocyst culture

Only about one embryo in 10 that is fertilised using IVF has the capacity to develop into a baby. Advances in culture techniques now allow scientists to culture an embryo to the blastocyst stage (50–100 cells), which usually occurs 5 days after fertilisation. With blastocyst culture, natural selection may pick those embryos that are more likely to implant and lead to pregnancy.

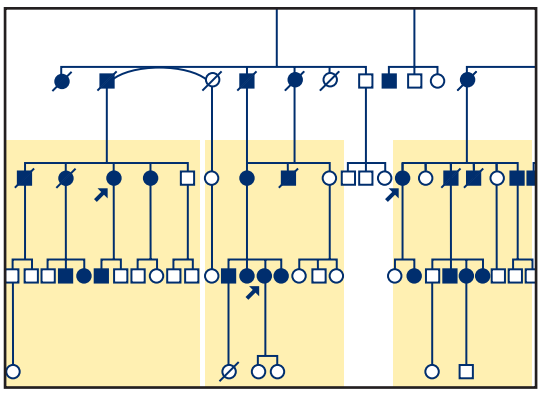

Figure 1. Representation of IVF. Sperm penetrating through zona

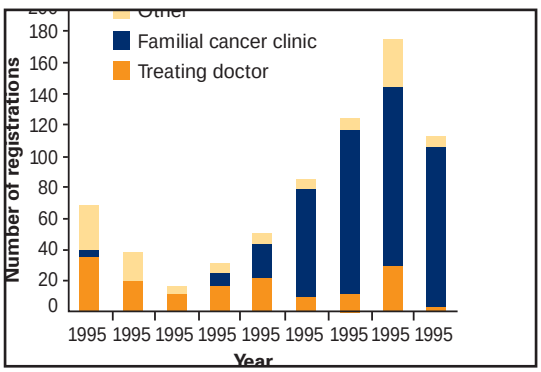

Figure 2. ICSI. Pipette (left) holding egg (centre) in position while sperm is injected with injecting pipette (right)

Assisted hatching

To facilitate implantation, a small breach in the outer layer of the zona (a thick transparent membrane surrounding the embryo) is made shortly before the embryo is placed in the uterus (*Figure 3*).

Surgical sperm collection

Surgical sperm collection is a collective term used for procedures that are available for men with tubal blockages and certain types of sperm production problems. Sperm collected by surgical procedures may then be used in conjunction with ICSI to achieve fertilisation.

Donor programs

A number of patients may require donor sperm, eggs or embryos in order to achieve a pregnancy. It is illegal in Australia to sell human gametes. Some fertility

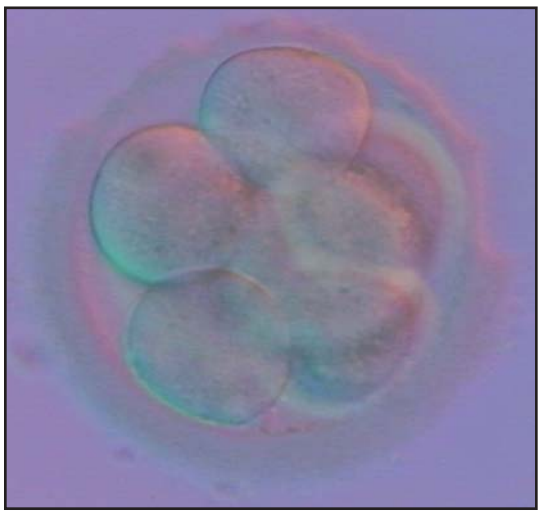

Figure 3. 'Hatching' – a small breach in the outer layer of zona for the embryo is made before transfer to assist implantation in the uterus

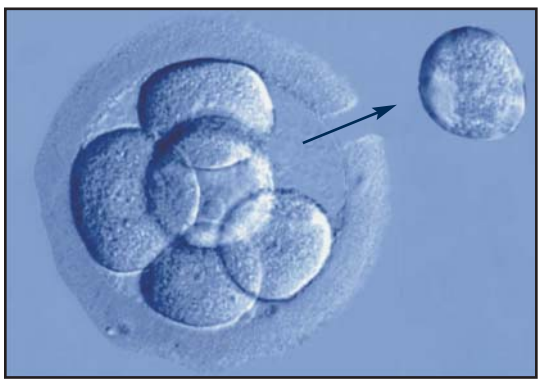

Figure 4. PGD. A cell is removed from the embryo 3–5 days after fertilisation to be analysed for a specific inherited disorder

clinics offer an extensive range of services to assist couples and ensure all legal, social and ethical issues are considered.

Genetics and pre-implantation genetic diagnosis

Pre-implantation genetic diagnosis (PGD) is the earliest form of prenatal diagnosis screening carried out on an embryo resulting from ART. It aims to prevent embryos carrying a genetic abnormality being transferred into the uterus. The woman undergoes an IVF-type procedure with ovarian stimulation to allow multiple egg collection. Fertilisation is carried out by ICSI and 3–5 days after fertilisation one or more cells are removed from the embryo. These cells are then analysed for a specific inherited disorder or chromosome abnormality (*Figure 4*). Results are available on day 5 following fertilisation and only healthy embryos are transferred into the uterus.

The same technique has been used for sex selection, however, the National Health and Medical Research Council recently recommended against PGD use for sex selection.[3] Pre-implantation genetic diagnosis is helpful if a couple has an increased risk of having a child with a specific genetic disorder, or for the couple finding it difficult considering the termination of an affected pregnancy after prenatal diagnosis at a later stage of pregnancy.

Worldwide, researchers are questioning whether PGD will allow improved embryo selection before embryo transfer, and hence improve pregnancy rates per cycle for all couples having IVF treatment. The studies performed so far have not been large enough or been designed to answer this question accurately.[4] At present, the cost to patients, as well as the limitation of the screening on each embryo and the accuracy of that screening, is minimising PGD's use. Studies are being carried out to understand and refine this technique further.

Conclusion

Subfertile couples in Australia have access to a range of ART techniques to assist them in conceiving, and ART techniques can also be used for applications such as pre-implantation diagnosis of genetic disorders. Improved laboratory procedure and embryo culture methods will have the most dramatic effect on improving pregnancy rates. In recent years, the outcome for pregnancies resulting from ART has improved with longer gestational age, higher birth weights and fewer perinatal deaths.

Conflict of interest: none declared.

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