

# A brief historical overview of assisted reproduction



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On 25 July 1978 Louise Brown was born. The event was surrounded by controversy, even moral condemnation; the world's first *in vitro* fertilisation (IVF) baby, following treatment by a uniquely British team – Patrick Steptoe, a district general hospital consultant in Oldham and Robert Edwards, a Cambridge don.<sup>1</sup> Since then assisted reproductive technology (ART) has developed at an extraordinary rate, resulting in the births of more than three million babies worldwide and achieving international public acceptance. Novel treatments for infertile couples have developed and IVF has become acceptable to patients and society.<sup>2-4</sup> The ease of treatment has improved, for example with the introduction in 1983 of vaginal aspiration of oocytes under ultrasound guidance, avoiding the risks and discomfort of laparoscopic oocyte recovery.<sup>5</sup> Some treatment advances have had spectacular results, such as the introduction of intracytoplasmic sperm injection (ICSI) in 1992, which revolutionised the management of male infertility.<sup>4</sup> Coupled with epididymal and testicular aspiration of sperm, ICSI has even allowed men with azoospermia to have their own genetic children.<sup>6-7</sup>

Adjustment to new practices in medicine is usually slow, yet no other field in medicine has integrated new knowledge into daily routine practice more quickly than ART. Many countries have introduced tight ethical regulation of ART to ensure good practice, protect patients and prevent unacceptable clinical practices and research, such as human reproductive cloning.<sup>8-9</sup> However, despite advances in ART the proportion of embryos leading to live offspring has increased only slowly since its inception, so there is still room for progress in terms of increasing healthy live births but decreasing multiple pregnancy rates.<sup>10</sup>

## Development of assisted reproduction techniques

### Alternatives to IVF and transcervical embryo transfer

Over the years IVF treatment has seen many modifications, and other options have been introduced. Prepared sperm may be introduced into the uterus by

intra-uterine insemination (IUI) at the time of ovulation, possibly following ovarian stimulation.<sup>11</sup> 1984 saw the first gamete intra-fallopian transfer (GIFT), in which oocytes are collected then introduced into the fallopian tubes together with prepared sperm at laparoscopy.<sup>2</sup> In 1986 zygote intra-fallopian transfer (ZIFT) was introduced; oocytes are collected and fertilised in the laboratory, then transferred into the fallopian tubes at laparoscopy.<sup>3</sup> As the availability and effectiveness of IVF has increased, other treatments such as GIFT and ZIFT have decreased, but IUI continues, although with some controversy regarding its true cost effectiveness.<sup>12</sup>

### Donor gametes, donor embryos and surrogacy

These treatment options are particularly sensitive in terms of acceptability by society and religions. Some religions such as Islam completely forbid them, and in many countries there is strict regulation of treatment. Although sperm cryopreservation in humans was introduced in 1953,<sup>13</sup> sperm donation commenced using fresh sperm in 1964. Later concerns of infection risks to recipients lead to the use of cryopreserved sperm to create sperm banks and quarantine sperm while donors were retested to exclude infections. In 1983 the first human pregnancy and birth after embryo donation fertilised *in vitro* was reported by Alan Trounson's group in Australia.<sup>14</sup> Surrogacy was first provided in 1985,<sup>15</sup> but to date remains a controversial area of treatment fraught with legal risks surrounding the handing over of the child from the birth mother to the commissioning parents.

### *In vitro* maturation of oocytes (IVM)

In 1965 Edwards confirmed the feasibility of IVM of immature human oocytes, but it was not until much later that this resulted in successful pregnancies.<sup>16</sup> The first pregnancy in a woman with anovulatory infertility following IVM of immature oocytes was reported by Trounson *et al.* in 1994.<sup>17</sup> In 1998 a birth was reported following cryopreservation of immature oocytes, thawing, IVM and fertilisation through ICSI.<sup>18</sup> Over 300 children have been born worldwide after IVM, with no reports to date of increased rates of malformation or disturbed development.<sup>19</sup>

## Semen assessment

The importance of male factor infertility assessment has become increasingly recognised. Discrepancies in the results of semen analysis (SA) can be caused by lack of standardisation of tests, so there is a clear need for internal quality control and external quality assurance programmes. The growing need to standardise procedures for the examination of human semen was acknowledged by the publication in 1980 of the World Health Organization (WHO)'s *Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction*.<sup>20</sup> Furthermore, organisations such as the WHO and the European Society of Human Reproduction and Embryology (ESHRE) now set up international training courses aiming at global standardisation.

## Micromanipulation of gametes and male infertility

Early work in this field using animal eggs started in the early 1980s. In the late 1980s Simon Fishel in the UK pioneered the technique of sperm subzonal injection (SUZI) in humans, involving injecting sperm under the zona pellucida (ZP),<sup>21</sup> leading to a pregnancy reported in 1988.<sup>22</sup> However, further work on this procedure had to be continued in Italy as a result of opposition in England. In the early 1990s ICSI was an experimental treatment<sup>4</sup> until perfected in 1992 by scientists in Brussels, led by Professor Andre van Steirteghem.<sup>6</sup> The widespread use of ICSI was accompanied by intense research into the indications, efficacy and safety of the procedure. In particular there has been close genetic scrutiny, as a genetic cause for male infertility may be passed on to the male offspring. In azoospermic men testicular sperm extraction has been used in combination with ICSI, to achieve fertilisation and pregnancy.<sup>7</sup>

## Pre-implantation genetic diagnosis (PGD)

In 1980 Alan Handyside in the UK<sup>23</sup> developed a method called PGD, to identify genetically abnormal embryos by testing individual cells, biopsied from embryos before their transfer to patients. The first successful birth followed within a few years and genetic testing techniques have developed markedly, including methods to show the X and Y chromosomes in a single human spermatozoon.<sup>24</sup> Although sex selection to avoid sex-linked inheritable diseases is valuable, there are ethical concerns regarding family balancing for social reasons.<sup>9</sup>

## Embryo culture and blastocyst transfer

*In vitro* embryo culture systems have evolved over the years since the pioneering work of Harrison in 1907. For human IVF, media are constantly being improved to be more biological,<sup>25</sup> mimicking the environment of the fallopian tube. Extended sequential culture media has allowed development of the embryo beyond 48 hours, for uterine transfer synchronous with normal physiology.<sup>26</sup> Transfer of the human embryo at the blastocyst stage (day 5 of development) was introduced in the early

1990s, leading to pregnancies in 1991.<sup>27</sup> Refinement in embryo culture has potential advantages, allowing better embryo selection, increased implantation rates and a decrease in multiple gestations by reducing the number of transferred embryos.<sup>28</sup>

## Cryopreservation of sperm

In 1949 glycerol was successfully used as a cryoprotectant for freezing bovine spermatozoa, leading in 1953 to successful pregnancies using human spermatozoa that had been frozen in dry ice.<sup>13</sup> In the early 1960s, Sherman described the first successful pregnancy using sperm that had been frozen with liquid nitrogen.<sup>29</sup> Sperm frozen in this way has now been used successfully for more than 40 years for both IUI and IVF. Freezing sperm may also preserve fertility prior to potentially sterilising treatments such as chemotherapy for cancer.

## Cryopreservation of embryos

The first human pregnancy achieved using embryo cryopreservation was reported in 1983, after modification of animal embryo cryopreservation techniques.<sup>30</sup> In the past 20 years, alternative protocols of embryo cryopreservation and thawing have been developed to optimise embryo survival and pregnancy rates.<sup>31</sup> Each country differs with regard to regulations and limits to storage periods. With such complex variations, patients occasionally need to seek judicial assistance to deal with their particular circumstances. The law in Britain, for example, limits the storage of embryos for longer than 5 years, but with specific consent this period can be extended to 10 years if using the patient's own gametes but not if using donor gametes.<sup>8</sup>

## Cryopreservation of eggs

Reports of live births from frozen oocytes have been increasing slowly since 1997.<sup>32</sup> However, only around 100 babies have been born worldwide using this technology.<sup>33</sup> If it becomes widely available, freezing of eggs could be offered to women facing premature loss of ovarian function, for example due to therapy for malignant disease. Oocyte cryopreservation avoids the ethical, logistic and legal issues associated with embryo freezing and donation, particularly if the woman does not have a partner.<sup>34</sup>

## Cryopreservation of ovarian tissues

Cancer therapy is increasingly effective today in terms of curing the disease but can irreversibly affect ovarian function, so preservation of fertility before therapy is important for women of reproductive age. Cryopreservation of human ovarian tissue received significant interest after the first successful animal study by Gosdon in 1994.<sup>35</sup> Human ovarian tissue may be cryopreserved with apparent good survival and function after thawing.<sup>36</sup> This procedure avoids the time delay due to the ovarian stimulation necessary before oocyte retrieval and cryopreservation of unfertilised or fertilised eggs, and the possible negative influences of ovarian stimulation on hormonally sensitive cancers.

An alternative option for preserving fertility is cryopreservation of immature oocytes (germinal vesicle, GV). The first successful cryopreservation of an immature human oocyte was reported in 1988.<sup>37</sup> GV recovered from stimulated ovaries showed significantly better maturation and developmental rates.<sup>37</sup> However, the cryopreservation procedures may have deleterious influences on chromatin and other organelles of the GV. In 1998 Tucker *et al.* reported on a birth after cryopreservation of GV, thawing, IVM and fertilisation through ICSI.<sup>18</sup>

### Assisted hatching

In view of speculation that some embryos may be unable to result in a pregnancy because they could not hatch from the encapsulating zona pellucida (ZP), assisted hatching was introduced in the late 1980s. In 1989 Cohen reported an increased implantation rate following mechanical opening of the ZP.<sup>38</sup> In 1990 Cohen reported an improved outcome after zona drilling with acid Tyrode's medium.<sup>39</sup> More recently, AH using laser photo ablation has also been encouraging.<sup>40</sup>

### Development of gonadotrophins for clinical use

Gonadotrophin preparations of varying composition and purity have been used to promote fertility over the past 40 years. In 1957 extracts of human pituitary gonadotrophins (hPG) containing both FSH and LH were administered for ovulation induction.<sup>41</sup> In 1988 hPG were abandoned due to safety concerns, when products derived from human pituitaries were linked with the development of Creutzfeldt-Jakob disease (CJD). In the early 1960s, the first human urinary gonadotrophins from postmenopausal females were licensed; later these preparations were purified and successfully introduced into clinical practice on a large scale. In 1963, Lunfeld had reported the first successful induction of ovulation followed by a pregnancy.<sup>42</sup> These earlier biological preparations had a high percentage of unknown urinary proteins, which interfered with batch-to-batch consistency, and being foreign proteins, they could induce the formation of antibodies resulting in painful skin rashes. Later highly purified urinary gonadotrophins were developed that had less contamination and hence less risk of such reactions. In 1995, genetically engineered gonadotrophins, the recombinant follicle-stimulating hormones, follitrophin alpha (GONAL-f, Serono International SA) and follitrophin beta (Puregon, Organon), were introduced into ART. These are devoid of LH activity, and structurally and biochemically are almost indistinguishable from each other.<sup>43</sup> However, a fill-by-mass (FbM) manufacturing process led to a new GONAL-f FbM preparation (Merck Serono International SA) that provides equivalent efficacy but improved consistency compared with the GONAL-f preparation obtained with the prior traditional fill-by-bioactivity process.<sup>44</sup>

### Progress in ovarian stimulation monitoring

Since the birth of Louise Brown in 1987 when an egg was collected from a natural cycle and fertilised *in*

*vitro*, natural cycle IVF has gradually been abandoned in favour of superovulation as a means of improving success of ART. The aim of producing multiple but controlled follicles is to collect multiple oocytes so that the best fertilised, cleaved embryos can be selected for transfer into the uterus.

### GnRH agonists and antagonists in ART

In 1971, Schally isolated and chemically characterised the structure of gonadotrophin-releasing hormone (GnRH).<sup>45</sup> Further work resulted in the formation of GnRH agonists, which cause brief pituitary stimulation followed by pituitary desensitisation. Use of GnRH agonists in the treatment of infertile women in 1982 stemmed from the pioneering work of Fleming.<sup>46</sup> In 1984, the use of GnRH agonists with gonadotrophins prior to IVF was first described by Porter *et al.*<sup>47</sup> The GnRH analogues have been shown to prevent premature luteinisation, decrease cancellation rates, increase the number of follicles and facilitate patients scheduling for oocyte retrieval. Refinement in GnRH antagonists continued after 1984, initially hampered by histamine release side-effects, which were overcome in 1994 with the third-generation GnRH antagonists that are currently in clinical use but still under assessment with regard to efficacy.<sup>48</sup>

### Luteal phase support during ART

Although the incorporation of GnRH agonists into IVF ovarian stimulation regimens was associated with improved outcome, this could result in luteal phase insufficiency as pituitary function takes time to recover completely after the end of GnRH-agonist therapy. Initially, human chorionic gonadotrophin (hCG) was used for luteal support, but because of the risk of ovarian hyperstimulation syndrome (OHSS), progesterone has become the agent of choice and has shown a positive impact on pregnancy rates.<sup>49</sup>

### Implantation

Although in most ART cycles apparently normal embryos are replaced, the majority fail to implant. To overcome low implantation rates multiple embryos are commonly replaced to increase the pregnancy rate. This has led to multiple pregnancies, which have significant risks. A very hot issue in IVF is how to reduce multiple births. Many professional and regulatory bodies are stressing the need to restrict the number of embryos transferred.<sup>50</sup> The Scandinavian experience organised by the Nordic Reproductive Health Council advocated single embryo transfer (SET), demonstrating that, by replacing only one embryo, twins can be avoided without major impairment of pregnancy rates. This approach has been endorsed by many.<sup>50</sup> Moving to SET, however, demands greater understanding of the development of human embryo *in vitro*, in order to select the best embryo for transfer, and careful patient counselling.

### Reproductive cloning

Cloning refers to the production of a second individual identical to the original organism. By somatic cell transfer

a differentiated cell may return to a totipotent stage, for instance by transferring the nucleus of a somatic cell into an enucleated oocyte. The reconstructed embryo could then develop in a surrogate mother. The birth of Dolly the sheep was an important milestone in the field of ART; it was the first proof that a differentiated cell could be reprogrammed to allow cloning of a new individual. In view of high incidence of developmental abnormalities associated with cloning and its moral implications most countries have introduced legislation against human reproductive cloning.<sup>9</sup> In contrast, non-reproductive use of such technology may produce human embryonic stem cells that could provide new therapies for diverse medical problems.<sup>51</sup>

## Regulation of ART

Although the introduction of IVF in 1978 caused an ethical and biological revolution, the British government was initially reluctant to introduce legislation. In 1984 the Warnock committee established a licensing authority to regulate infertility treatment with an arrangement for licensing practices and researchers. In 1991 the British Human Fertilisation and Embryology Act set out a framework for ART practice and research under licence from the Human Fertilisation and Embryology Authority (HFEA). In many developed countries, regulatory bodies have been introduced to legislate ART in response to public concern about its implications and the effect it might have on the value of human life and family relationships.<sup>8</sup>

## Conclusion

At the start of a new millennium, reproduction is without doubt one of the most dynamic developing fields in human medicine. In spite of ever-increasing knowledge and skills many questions remain unanswered, and new concerns and challenges constantly arise. ART must therefore be applied responsibly with the highest regards for human dignity. Rather than crude pregnancy rates, the birth of a healthy singleton baby is increasingly becoming the most important outcome parameter following assisted reproductive treatment.<sup>52</sup>

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