In-vitro fertilization

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Abstract

Most young couples trying for a baby will eventually conceive within 18 months and only one in seven couples in the UK remain subfertile after this period. In 80% of these subfertile women one or more causative factors are attributable; and some may be associated with significant co-morbidities. Even when no identifiable physical disease is revealed, the psychological impact on affected couples can be severe; and approximately 50% of subfertile couples will eventually undertake some form of assisted conception treatment. In-vitro fertilization (IVF) can be viewed both as a test of reproductive potential, allowing detailed assessment of oocytes, oocyte–sperm interaction and embryo quality, as well as an effective treatment for most forms of subfertility. The dramatic improvements in pregnancy rates seen with IVF treatment since its inception some 34 years ago have occurred due to close multidisciplinary collaboration and the practical application of scientific advances in embryology and pharmacology. There have been several important landmarks including: the use of drugs for superovulation and pituitary downregulation; the introduction of transvaginal ultrasound for monitoring of follicle growth and oocyte retrieval; developments in embryo culture; oocyte donation; and the introduction of intracytoplasmic sperm injection for the treatment of severe forms of male infertility. In the past decade, we have also witnessed the development of new technologies, including pre-implantation genetic diagnosis, the in-vitro culture of immature oocytes to viability, and the cryopreservation of oocytes, thereby widening the scope of clinical problems that can be addressed by IVF-associated technologies. Despite this progress, the majority of IVF cycles still do not produce a viable pregnancy. The psychological stresses imposed upon couples by assisted conception treatment need to be managed carefully and sympathetically. IVF practice continues to require support from appropriately trained and skilled counsellors.

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Introduction

Following years of research and collaboration between Robert Edwards, a scientist from Cambridge and Patrick Steptoe, a gynaecologist in Lancashire Louise Brown, the world's first baby to be conceived outside the human body, was born in Britain in July 1978. This collaboration was essential as Steptoe was able to collect the oocytes by transabdominal needle aspiration of mature follicles under laparoscopic view and Edwards had the necessary laboratory skills to achieve the growth of embryos after fertilization *in vitro*. The importance of collaboration between disciplines remains as true today as it was in the 1970s. The birth of Louise Brown was soon followed by the birth of further in-vitro fertilization (IVF) babies in Australia and the United States (US). At this time, there was considerable disquiet among both the public and the medical profession concerning the morality, and even legality, of the process of IVF. This debate led to the establishment first of a Voluntary Licensing Authority, and later the Human Fertilisation and Embryology Authority (HFEA), by the UK government. The role and powers of the HFEA continue to change as the umbrella of IVF applications continues to grow; and the organization has been emulated in many countries over the past two decades.

How IVF works

Although 'human' IVF did not come of age until 1978, most of the technological steps needed for Louise Brown's conception had been developed years earlier in veterinary medicine. The principle of IVF is simple: collect mature oocytes (eggs) from the ovaries, fertilize them in cell culture in the laboratory and replace embryo(s) into the uterine cavity at such a time in the cycle to ensure a receptive endometrium. However, each step in this model has been refined by decades of experimentation, leading to the quasi- industrial IVF of today.

Stimulation of multiple follicle development

One of Steptoe and Edwards greatest problems was a shortage of oocytes. They were able to harvest only one or two oocytes in the natural cycle, and frequently, by the time that laparoscopy had been arranged, general anaesthesia induced and surgery undertaken, the follicle had ovulated and the oocyte had been lost in the pelvis. Success rates were, therefore, low. In order to improve the chances of having embryos for transfer, the embryologist needed more oocytes and the clinicians needed to be certain that premature ovulation would not occur.

Gonadotrophins

Gonadotrophins synthesized and secreted by the pituitary gland play a central role in regulating the ovarian cycle of follicular recruitment, selection and maturation, oocyte release and corpus luteum development and regression. Exogenous gonadotrophins have been used for many years to induce ovulation in anovulatory patients.

Gonadotrophins were first demonstrated to be present in the urine of pregnant women by Ascheim and Zondek in 1927. Three years later, Zondeck reported their presence in the urine of menopausal women. However, the first use of gonadotrophins for inducing multiple ovulations (superovulation) utilized extracts of human pituitary tissue collected at post-mortem. Scarcity of post-mortem pituitary glands made this source impracticable; in any case, by the 1980s, pituitary derived gonadotrophins were found to have transmitted Creutzfeldt–Jakob infection in a small number of cases. In 1947 human menopausal gonadotrophins were first extracted from urine in pharmacological amounts, creating the possibility of a readily available pharmaceutical source for widespread clinical use. More recently, recombinant follicle-stimulating hormone (FSH) and luteinizing hormone (LH) have been made available following transfection of the genes for the alpha and beta chains of human FSH or LH into Chinese hamster oocyte cell lines, producing large amounts of glycosylated FSH and LH without the need for collection and extraction from human urine.

Treatment with gonadotrophins was initially by intramuscular injection of a relatively impure fraction containing both LH and FSH. In time, purified urinary FSH and later recombinant FSH became available for subcutaneous injection. These injections can be self-administered and are tolerated well by patients. The current era of fertility therapy focuses on simplifying treatment regimens by minimizing clinic attendances, and reducing the pain and stress of treatment, thereby improving compliance.

Gonadotrophin injection encourages the development of a cohort of mature follicles in a single stimulation cycle, overriding the physiological processes of follicle selection and dominance. Egg collection could yield anything up to 40 oocytes after 10–14 days of daily injection. In the early days of superovulation, ultrasound had not developed sufficiently for the assessment of follicle growth, and the only means by which clinicians could obtain an approximate measurement of ovarian response to treatment was by the daily measurement of urinary oestrogens, a process that was time-consuming and inaccurate. In addition, the rapid rises in circulating oestrogen levels were likely to trigger a premature LH surge, leading to ovulation before egg collection. Having established a means of obtaining multiple oocytes, the next development was to prevent the premature LH surge.

Gonadotrophin-releasing hormone agonists

Gonadotrophin-releasing hormone (GnRH) agonists have acquired an important place in IVF treatment. The decapeptide structure of GnRH was described in 1971. It was initially thought that agonists would be used as strong sustained stimulators of gonadotrophin secretion. It soon became evident that administration of these agents, after an initial bolus release of gonadotrophins, suppresses LH and FSH levels by pituitary receptor downregulation and desensitization. In IVF treatment, this prevents the occurrence of a premature LH peak in response to rising oestradiol levels from the growing follicles, preventing spontaneous ovulation and allowing planned oocyte retrieval. However, since the effect of ovarian quiescence is produced at the pituitary, injections of FSH hormone would still stimulate the ovary to resume folliculogenesis. Hence, from the mid-1980s, it became common-place to begin an IVF treatment cycle with 'pituitary downregulation', followed by ovarian stimulation with gonadotrophin injection after downregulation had been confirmed by the measurement of low levels of oestradiol and LH in the circulation, and endometrial shedding confirmed by the ultrasound demonstration of a thin endometrium. This so-called 'long protocol' was followed by a number of variations on the theme. All share the same application of GnRH agonist to avoid a premature LH surge. In each protocol, a timed LH surge is created by a single injection of human chorionic gonadotrophin (hCG). This is essential to induce the oocyte to re-enter meiosis and extrude the first polar body and to begin the process of luteinization of the follicles, which prepares the endometrium for implantation.

GnRH analogues with antagonist properties

An alternative to GnRH agonists are GnRH antagonists such as Cetrorelix or Ganirelix. GnRH antagonists directly block the GnRH receptor, isolating it from the action of native GnRH, without an initial stimulation, thereby avoiding the 'flare' effect. This has a number of potential benefits. Since the flare is avoided, duration of treatment for controlled superovulation may decrease. The total dose of FSH required for treatment may be reduced. Use of GnRH antagonists may reduce the incidence and severity of ovarian hyperstimulation states associated with superovulation. Unless GnRH antagonists are administered for a prolonged period of time, pituitary downregulation does not occur, making them rapidly reversible. The 'antagonist' IVF cycle begins with the injection of FSH from day 1 or 2 of the menstrual period, with an addition of GnRH antagonist a few days later to prevent an LH surge. FSH injection is continued until hCG is given to mimic a timed LH surge. In GnRH antagonist IVF cycles, where there is a significant risk of ovarian Hyperstimulation Syndrome (OHSS), LH surge and oocyte maturation can be safely induced with a single injection of GnRH agonist instead of hCG.

The use of GnRH antagonists allows a more rapid IVF treatment cycle without the need for preliminary downregulation, avoiding the troublesome menopausal side-effects of downregulation. Ovarian cyst formation that is seen in up to 10% of 'long protocol' cycles due to the 'flare' effect of raised FSH on the ovary are avoided by the use of GnRH antagonists.

Monitoring of follicle growth

Accurate monitoring of follicle growth during ovarian stimulation is essential both for the correct timing of hCG administration (a follicle contains a mature oocyte when it reaches 17–18 mm diameter) and to avoid ovarian hyperstimulation by discontinuing FSH injection if too many follicles begin to develop. In older women, monitoring can also predict 'poor response', failure to develop growing follicles despite FSH injection. Such patients can again be advised to stop treatment or can at least be warned that pregnancy is unlikely if they continue stimulation and proceed with egg collection.

The growth of ovarian follicles can be monitored either directly by visualization with ultrasound or by measuring their output of oestradiol into the circulation. The widespread availability of transvaginal ultrasound has facilitated follicle monitoring considerably – the procedure is painless and gives accurate assessment of follicle diameters and endometrial