INTRODUCTION

Most of the estimated 5 million babies born worldwide to IVF treatment are products of ovulation induction (Human Fertilisation and Embryology Authority, 2011). However, the application of ovulation induction to IVF was fraught with problems that had to be overcome before it became the global infertility treatment it is today.

Robert Edwards and his clinical partner, Patrick Steptoe initially relied on human menopausal gonadotrophin (HMG) and human chorionic gonadotrophin (HCG) as ‘priming hormones’ when they began their early IVF trials on infertile women. However, repeated pregnancy failures using oocytes from scores of gonadotrophin-stimulated women pointed them in a different direction. The first successful human IVF pregnancy eventually came from a natural menstrual cycle, involving intensive endocrine monitoring to track pre-ovulatory follicular development and collect a single ripe egg timed to the natural LH surge. Despite this groundbreaking achievement, ovulation induction was clearly required to make IVF treatment clinically robust and reliable. Ovarian stimulation with clomiphene citrate was used to achieve the first maternity from a superovulated human IVF cycle in 1980. HMG/HCG regimens were then successfully introduced—including substitution of ‘pure’ follicle-stimulating hormone as the principal ovarian stimulant. The application and success of IVF treatment were dramatically enhanced by the introduction of gonadotrophin-releasing hormone analogues that enabled elective control of endogenous gonadotrophin release during ovarian stimulation. Programmed gonadotrophin regimes yielding double-digit oocyte numbers became normal: ‘more is better’ was the ethos. Bob Edwards expressed increasing concern over the cost, complexity and potential long-term health risks of such high-order ovarian stimulation. In later life he repeatedly called for a return to minimalist approaches based on the natural menstrual cycle to improve oocyte quality over quantity. This article reviews the application of ovulation induction to human IVF and celebrates Edwards’ abiding impact on the field, which firmly grounds him in the reproductive endocrinology pantheon.

Key words: endocrinology / gonadotrophins / oocyte quality / ovarian follicle / fertilization

HORMONE PRIMING

We started cautiously, using low amounts of hormones to stimulate the ovary and then a touch of HCG to induce the ripening programme. (Edwards, 1980a)

The first attempts to collect pre-ovulatory oocytes suitable for IVF used HMG ‘to impose some control over the menstrual cycle and to stimulate the ripening of the eggs in the ovary’ (Steptoe and Edwards, 1970). Edwards had overturned the dogma that adult ovary was resistant to gonadotrophins, using mice treated with pregnant mare serum gonadotrophin and HCG (Fowler and Edwards, 1957). Steptoe (1968) had witnessed ovulation in gonadotrophin-treated women through his laparoscope. It was therefore entirely logical to use ‘hormone priming’ towards recovering human pre-ovulatory oocytes to attempt IVF. Little was then known about methods of ovarian stimulation in cyclic women. Treatment with gonadotrophins had been reserved for amenorrhoeic women with the aim of achieving natural conception through the fertilization of a single ovulated oocyte. However, multiple ovulation was a frequent response and the chance of conceiving triplets or more was 1 in 10 (Gemzell, 1966).

The hormone priming protocol used to provide proof-of-concept for laparoscopic oocyte recovery was entirely empirical. It began with 150 IU HMG given once early in the menstrual cycle. This was gradually increased to two or three injections of 225 IU HMG given 2–3 days

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apoart over Days 2—9. To induce ovulation, one injection of HCG (1000—12 500 IU) was given between Days 9 and 11, preferably on Day 10. Laparoscopic oocyte collection was done the next day, 29—31 h after giving HCG. In their own words, ‘HCG was given at an arbitrary time, selected for our own purposes and not because the patient had been fully primed by the HMG’. Thus IVF endocrinology was born a decade before the first IVF baby.

The landmark Steptoe and Edwards (1970) paper established:

- Follicle development and oocyte maturation can be controlled in cyclic women by the use of HMG and HCG.
- A regimen of three injections of HMG (225 IU per injection) between days 2 and 9, and 5000 IU HCG on Days 9—11 of the menstrual cycle gave the best response.
- Oocytes could be aspirated laparoscopically from their Graafian follicles, and approximately a third of the aspirated follicles yielded an oocyte.

Oocytes sourced in this way were capable of fertilization and cleavage to the morula stage of embryonic development. However, none of the first 77 patients to whom embryos were replaced after staged treatment with HMG and HCG experienced a successful pregnancy (Edwards et al., 1980).

Tactics changed after a series of false starts including an ectopic pregnancy following uterine replacement of an IVF embryo generated from an HMG/HCG primed cycle (Steptoe and Edwards, 1976). This patient had received 900 IU HMG, resulting in the collection of four oocytes. One of these had fertilized and developed into the embryo that implanted ectopically.

Natural cycle

...goodbye to the fertility drugs... ‘There was no alternative but to collect the single egg ripening naturally within the mother.’ (Edwards, 1980b)

Increasingly concerned that the hormones used to stimulate pre-ovulatory follicles before oocyte collection might be having deleterious effects on luteal or uterine function, Steptoe and Edwards turned to the spontaneous menstrual cycle. Many of the HMG/HCG cycles they reported had shortened luteal phases, and they discovered a strong negative association between follicular phase urinary estrogen levels and luteal phase duration. With hindsight it seemed, ‘endocrine disturbances following the use of HMG and HCG to induce follicular development and luteinization in cyclic women were perhaps too much to overcome, and this method may have been abandoned permanently in favour of monitoring the natural menstrual cycle’. (Edwards et al., 1980).

This decision seems all the more heroic given the primitive nature of clinical hormone testing in the 1970s. Reliable methods for LH determination by radioimmunoassay were a distant prospect and estrogen immunoassay was still in its infancy. The assays available were often stretched to the limit of their sensitivity, and produced results that were difficult to interpret.

The follicular phase of the natural menstrual cycle was monitored by measuring urinary levels of oestrone glucuronide (24 hourly) and LH (3 hourly) to time oocyte aspiration. Since estrogen concentrations typically rise until day 13 of the menstrual cycle, it was reasoned that the estrogen assays would give the amber light for egg collection, which could be fine tuned by LH testing using the Hi-Gonavis immuno-precipitation assay for HCG that recognized LH (Edwards et al., 1982). This last would soon be replaced by specific LH radioimmunoassay (Kerin et al., 1980). Edwards and Steptoe had already determined that optimal timing for follicle aspiration was 32—34 h after the injection of HCG (Edwards and Steptoe, 1975). They therefore reasoned that it would be somewhat less after the accumulation of LH in urine at a level detectable by the Hi-Gonavis test. All four pregnancies resulting from the first series of natural cycles came from oocytes aspirated 24 h or longer after the LH surge began. One of these pregnancies progressed to term as the first ever IVF pregnancy (Steptoe and Edwards, 1978).

This experience validated the prospect of establishing human term pregnancies following the aspiration of oocytes, their fertilization in vitro and the placement of a cleaving embryo into the mother’s uterus. IVF and its many variants such as gamete intra-fallopian transfer (GIFT), ICSI, etc. would rapidly become the treatment of choice for many if not most forms of female and male infertility.

Crucially, clinical IVF success had come about through monitoring spontaneous menstrual cycles and working with the naturally ripened ‘gold-standard’ oocyte (Fig. 1). The implication was that for ovulation induction to succeed, new tactics were required to take account of the physiological realities of the normal ovarian cycle.

Controlled stimulation cycles

...there is an advantage in replacing two or more embryos in the uterus’. (Edwards and Steptoe, 1983)

The second (Steptoe et al., 1980) and third (Lopata et al., 1980) IVF births in the world also came from natural cycles. However, the logistics were overwhelming due to the absolute need for intensive endocrine monitoring and random access to operating theatre for egg collections dictated by spontaneous LH surging, and the technical challenge of working with individual oocytes and embryos (Kerin et al., 1984). It was also evident from animal breeding experience that working with multiple eggs and embryos should increase the likelihood of clinical IVF success (Trounson and Leeton, 1982). So the quest for a successful stimulation protocol continued.

A second wave of practitioners persisted with clomiphene (Trounson et al., 1981) (see below), HMG (Wortham et al., 1983a) and combinations of the two (Vargyas et al., 1984). The first IVF successes with HMG were achieved with ‘individualized’ therapy using 150—225 IU HMG injected daily for variable periods beginning on Days 2 or 3 of the menstrual cycle (Trounson et al., 1980) and third (Lopata et al., 1980) IVF births in the world also came from natural cycles. However, the logistics were overwhelming due to the absolute need for intensive endocrine monitoring and random access to operating theatre for egg collections dictated by spontaneous LH surging, and the technical challenge of working with individual oocytes and embryos (Kerin et al., 1984). The first IVF successes with HMG were achieved with ‘individualized’ therapy using 150—225 IU HMG injected daily for variable periods beginning on Days 2 or 3 of the menstrual cycle (Trounson et al., 1980). The dose and duration of HMG treatment were varied according to ultrasonic measurements of follicle size and number as well as estrogen testing in blood or urine. Typically, HMG would be stopped once multiple estrogen-secreting follicles were detectable and HCG injected 2—3 days after the last HMG injection. An average of up to 4 pre-ovulatory oocytes could be collected in this way. Oocyte maturity was variable and ovarian hyperstimulation syndrome (OHSS), a constant threat (Acosta et al., 1984). Importantly, clinical success also came using immature oocytes that were fertilized after completing maturation in vitro (Veeck et al., 1983; Wortham et al., 1983b).

More aggressive ‘high-dose’ HMG therapy also succeeded. A minimum of 225 IU (three vials) HMG was given daily for 5 days starting on Day 3 of the cycle (Lauffer et al., 1983). HMG dose was gradually raised to 400 IU per day for a further 1—4 days, depending on the response.
HCG was given when at least two large follicles between 16 and 18 mm in diameter were visualized on ultrasound. At least 10 oocytes would usually be collected but follicular synchrony was poor and uterine receptivity disturbed (Laufer et al., 1984b) (Fig. 1).

The limitations to HMG/HCG protocols gradually became better understood, casting light on the earlier failures experienced by Edwards and Steptoe. The amplitude of the spontaneous LH surge may be reduced and the timing of its onset altered by daily injection or pulsatile administration of HMG (Afnan et al., 1984; Glasier et al., 1988; Ferraretti et al., 1983). The decision when to give HCG becomes pivotal if the LH surge is suppressed, since multiple asynchronous oocytes can be recovered timed to HCG or natural LH surge. NB: the LH surge may be delayed. (B) Clomiphene + HMG/FSH: giving additional FSH-containing gonadotrophin extends the window of endogenous stimulation, causing more subordinate follicles to enter pre-ovulatory development. Up to 6 or more relatively synchronous oocytes can be expected, with recovery timed to HCG or natural LH surge. NB: the LH surge can be abnormal. (C) HMG/FSH: FSH-containing gonadotrophin preparations augment endogenous FSH and LH. Standard regimens drive multiple subordinate follicles into pre-ovulatory stages of development. Ten or more oocytes of variable quality can usually be recovered timed to HCG or natural LH surge. NB: the LH surge is frequently abnormal. (D) GnRH agonist + HMG/FSH: GnRH agonist given from the previous mid-luteal phase (‘long’ GnRH agonist protocol) suppresses endogenous FSH and LH until only precursor follicles remain. HMG or FSH treatment builds an artificial follicular phase in which multiple intermediate follicles are impelled into advanced stages of development. More than 15 oocytes of variable quality are normally recoverable timed to injection of HCG. NB: the natural LH surge is blocked due to pituitary desensitization.

Figure 1 IVF ovulation induction: protocols explained. Ovulation induction is constrained by the innate follicle–follicle difference (i.e. asynchrony) that underpins mono-ovulation (Hillier et al., 1985). No two follicles are ever the same, nor the oocytes within them. Follicular maturation in the normal menstrual cycle can be depicted as a triangle with the fully mature follicle (≥25 mm diameter) at its apex and the cohort of precursor follicles (<5 mm diameter) from which it emerges at its base, with intermediate stages between. Cyclic fluctuations in blood FSH and LH levels are insufficient to prevent all but the dominant follicle (coloured gold) regressing by mid-cycle. (A) Clomiphene: clomiphene treatment causes additional FSH and LH release that stimulates ≥2 more pre-ovulatory follicles (white arrows). Normally ≥3 (white numerals) relatively synchronous oocytes can be recovered timed to HCG or natural LH surge. NB: the LH surge may be delayed. (B) Clomiphene + HMG/FSH: giving additional FSH-containing gonadotrophin extends the window of endogenous stimulation, causing more subordinate follicles to enter pre-ovulatory development. Up to 6 or more relatively synchronous oocytes can be expected, with recovery timed to HCG or the LH surge. NB: the LH surge can be abnormal. (C) HMG/FSH: FSH-containing gonadotrophin preparations augment endogenous FSH and LH. Standard regimens drive multiple subordinate follicles into pre-ovulatory stages of development. Ten or more oocytes of variable quality can usually be recovered timed to HCG or natural LH surge. NB: the LH surge is frequently abnormal. (D) GnRH agonist + HMG/FSH: GnRH agonist given from the previous mid-luteal phase (‘long’ GnRH agonist protocol) suppresses endogenous FSH and LH until only precursor follicles remain. HMG or FSH treatment builds an artificial follicular phase in which multiple intermediate follicles are impelled into advanced stages of development. More than 15 oocytes of variable quality are normally recoverable timed to injection of HCG. NB: the natural LH surge is blocked due to pituitary desensitization.
then be injected in the absence of LH or combined with HMG to increase the FSH:LH ratio (Jones et al., 1984, 1989; Muasher et al., 1985).

In the Norfolk IVF programme that produced the first American IVF birth in 1981, blood FSH/LH ratio was measured on Day 3 of the menstrual cycle to predict the likelihood of a patient’s response to a standard HMG protocol. In patients with a high FSH/LH ratio (i.e. potential poor responders) substitution of high-dose uFSH for HMG improved oocyte recovery, embryo numbers and pregnancy rate (Jones et al., 1989). We now realize that poor responsiveness to FSH can be associated with a diminished ovarian reserve (i.e. number of FSH-responsive follicles) and/or the presence of FSH receptor isoforms that cause FSH insensitivity (Simoni et al., 2002). The size of the ovarian reserve reduces with age and can be influenced by genetics, previous chemotherapy, etc. High-dose FSH remains a treatment option for poor responders but the prognosis tends to be poor (Oudendijk et al., 2012).

**Clomiphene citrate**

Edwards and Steptoe had tried and abandoned clomiphene and tamoxifen in their early IVF trials (Edwards et al., 1980). However, others had persisted, showing that clomiphene could be used with either HCG or natural LH release to achieve full-term IVF pregnancies (Johnston et al., 1981; Trounson et al., 1981). The basic regimen was oral clomiphene (50–150 mg per day) for up to 5 days, beginning early (normally Day 2 or 5) in the follicular phase, with egg collection timed to the spontaneous LH surge or HCG injection (Marrs et al., 1984; Quigley et al., 1984a).

Edwards was quick to acknowledge that the reintroduction of clomiphene by the Australians had moved forward IVF treatment considerably (Edwards, 1982). He and Steptoe were encouraged to return to clomiphene with startling success. ‘Between October, 1980, and April, 1983, embryos fertilized in vitro were replaced in the uteri of 1200 women. The clinical pregnancy rate rose from 16.5% from October, 1980, until September, 1982, to almost 30% in 1983. The proportion of pregnancies ending in abortion varied from 25–35%. Factors favourably modifying implantation rate were maternal age of under 40 years, priming with clomiphene alone, and the replacing of more than one embryo in the uterus. The replacing of two or more embryos, increasing maternal age, a poor obstetric history, and high levels of follicular estrogens raised the chances of abortion’ (Edwards and Steptoe, 1983). Their early results with clomiphene alone were better than those after no treatment or after clomiphene/HCG, indicating that the natural LH surge following clomiphene resulted in embryos or uteri that were superior for implantation.

Disadvantages with clomiphene are that the spontaneous LH surge is occasionally blocked so precise timing of HCG administration is important to avoid cystic or prematurely luteinised follicles. Anti-estrogenic effects on the uterus may also be detrimental to embryo implantation (Hillier et al., 1985).

As a selective estrogen receptor modulator, clomiphene is a racemic mixture of the two stereoisomers zuclophenone and enclomiphene (Goldstein et al., 2000). The active agent is the anti-estrogenic en isomer, which enhances pituitary FSH and LH secretion through estrogen-receptor blockade in the hypothalamo-pituitary axis (Goldstein et al., 2000). The clomiphene en isomer is more powerful than the zuc in stimulating follicular development (Glasier et al., 1989) and is a potential ovarian stimulant in its own right (Adashi, 1993). Highly estrogenic zuclophenone is likely to be responsible for the premature LH surges that occasionally occur during clomiphene therapy.

Clomiphene remains an attractive option. Cheap and conveniently administered as tablets, it stimulates endogenous gonadotrophin levels and produces multiple (typically 2–3) relatively synchronous follicles (Fig. 1). There is a reasonably tight relationship between pre-ovulatory follicle number determined by ultrasound and blood estradiol levels in clomiphene cycles. Algorithms based on these parameters can be used to optimize the timing of HCG injection (Trounson and Leeton, 1982; Hillier et al., 1984).

**Clomiphene plus HMG**

Before fully programmable ovarian stimulation was realized (see below), combined or sequential treatment with clomiphene and HMG or ‘pure’ uFSH became increasingly popular. During 1980–1983 clomiphene/ HMG/HCG was the most common successful stimulation protocol used in Australia (Lopata, 1983; McBain and Trounson, 1984). A typical regime would be clomiphene (100 mg) daily from Days 2 to 6 of the menstrual cycle, followed by HMG or uFSH (150–225 IU) from Day 5, with estrogen assays and follicular ultrasound to monitor the ovarian response, and a 1–2 day ‘coast’ before administering HCG (Okamoto et al., 1986). Simultaneous or sequential clomiphene/HMG therapy usually stimulates more pre-ovulatory follicles than clomiphene alone but produces a smaller cohort of more synchronous follicles than HMG or FSH alone (Hillier et al., 1985) (Fig. 1). Simultaneous therapy is advocated for patients who do not respond satisfactorily to the sequential regime (Kerin et al., 1984; Quigley et al., 1984b).

Treatment regimes combining clomiphene and HMG or uFSH are generally equally effective in inducing multiple follicular development in normal women (Messinis et al., 1996) and would nowadays be classified as minimal or mild IVF stimulation protocols (Teramoto and Kato, 2007).

Controlled ovarian stimulation allowed the development of IVF into the highly successful therapeutic process we know today, but in truth it was far from ‘controlled’. Which stimulation regimen to use? How to monitor ovarian response? When to predict oocyte collection? How to avoid OHSS? These problems would be offset, and new ones posed, by the introduction of programmed stimulation cycles using gonadotrophin-releasing hormone (GnRH) analogues, as discussed below.

**Programmed stimulation cycles**

Programming protocols may help to organize the activities of the clinic, but primary emphasis should be placed on the best interests of the patient’. (Edwards et al., 1996)

The use of oral contraceptives to control the menstrual cycle preceding ovarian stimulation was an early step towards programming oocyte collection for IVF (Frydman et al., 1986; Wardle et al., 1986).

However, IVF logistics were more profoundly influenced by the introduction of GnRH ‘super’ agonists to down-regulate endogenous FSH and LH secretion during staged treatment with HMG and HCG (Fleming et al., 1982; Porter et al., 1984; Rutherford et al., 1988). This made it feasible to override cyclic ovarian function and conduct programmed ovarian stimulation without routine LH and estrogen testing (Golan et al., 1994). The natural LH surge being stopped, ultrasound measurements alone were sufficient to monitor follicular responses and time HCG (Lass, 2003). This caused a step change in the applicability and convenience of IVF and related assisted reproductive technology treatments (Barbieri and Hornstein, 1999; Felderbaum and Diedrich, 1999). It cannot pass
unmentioned that associated advances in ultrasonography to monitor follicular status and direct oocyte retrieval hugely impacted this development and remain vital to the management of all types of IVF therapy today (Wikland et al., 1988).

IVF stimulation regimes involving GnRH analogues have now been mainstream for a quarter of a century (Frydman et al., 1988). Most practitioners continue to combine HMG, uFSH or recombinant (rec) FSH (see below) with GnRH agonist to suppress endogenous FSH and LH secretion (Nardo et al., 2013). In the ‘long’ GnRH agonist protocol, daily (intranasal spray or SC injection) or long-acting depot GnRH agonist is started in the mid-luteal phase until ovarian follicular development ceases, based on ultrasonic assessment and estrogen measurements. Daily treatment with HMG or FSH then commences. Ovarian response is monitored ultrasonically, beginning ~8 days after starting gonadotrophin treatment. HCG is given when sufficient follicles of adequate size have developed. Blood estrogen assays are advocated to check the ovarian response, in case HCG need be withheld to avoid OHSS. HMG and uFSH are similarly effective in this type of regime (Westergaard et al., 1996; Bagtrée et al., 1998).

Alternative ‘short’ or flare-up GnRH protocols build on the capacity of GnRH agonists acutely to sustain endogenous gonadotrophin release before causing pituitary down-regulation. The initial burst of FSH and LH stimulates a wave of pre-ovulatory follicular development, which is then extended by treatment with exogenous gonadotrophin. Typically, daily therapy with a short-acting GnRH agonist is begun on Day 1 or 2 of the cycle with gonadotrophin (HMG or FSH) started on Day 3. Timing of HCG is based on ovarian follicular ultrasound backed up with estrogen testing, as described above (Tarlatzis et al., 1993; Marcus and Ledger, 2001).

Importantly, estrogen testing without ovarian ultrasonography is unreliable for monitoring responses to LH-free gonadotrophins in GnRH-agonist protocols, since results generally underrepresent the degree of follicular maturation on the day of HCG administration (Agrawal et al., 1998). Most units have long since abandoned all estrogen monitoring and rely on ultrasound alone.

Long GnRH agonist protocols involving stimulation with HMG or FSH treatment, are by far the most popular in current use for standard IVF purposes. However, they provide much more than the hormone priming initially envisaged by Bob Edwards. They switch off endogenous gonadotrophin release and depend entirely on exogenous input. The dose and duration of gonadotrophin treatment is generally geared to maximizing oocyte recovery in order to increase the number of oocytes available for treatment. The follicles that respond are therefore highly asynchronous and yield oocytes of variable quality (Fig. 1). A review of 400, 135 IVF treatment cycles in the UK revealed that recovery of up to 15 oocytes per collection is necessary to maximize the chance of a live birth from IVF therapy (Sunkara et al., 2011). Most of these cycles would have involved long GnRH agonist protocols employing HMG or FSH. A recent analysis of IVF live birth rates in 2455 Chinese women on long GnRH agonist protocols drew similar conclusions (Li et al., 2013).

GnRH antagonists capable of safely and effectively blocking LH release took more than a decade longer than GnRH agonists to be introduced into clinical practice (Howles, 2002; Olivennes et al., 2002). These drugs found immediate use in gonadotrophin-stimulated IVF cycles to block the natural LH surge and avoid premature luteinization (Felberbaum and Diedrich, 1999; Ganielux Dose-finding Study Group, 1998).

In the multiple dose GnRH antagonist protocol, 0.25 mg of antagonist is given daily SC beginning on Day 6 of gonadotrophin up to and including the day of HCG administration. Advancing or delaying the ‘optimal’ time of HCG administration by up to a day seems not to affect IVF live birth outcome adversely in this protocol, adding substantially to its convenience (Tremellen and Lane, 2010; Morley et al., 2012).

GnRH antagonist protocols permit the use of GnRH agonist to trigger final follicle maturation (via endogenous LH) instead of HCG. Pregnancy rates are lower compared with the use of HCG, which remains the clinical standard (Griesinger et al., 2006; Youssef et al., 2011a, b). Oral GnRH agonists are also being investigated for this purpose (Gerrits et al., 2013).

Three decades after the first reported successful IVF stimulation protocol involving a GnRH analogue, the evidence base continues to grow. Long GnRH agonist protocols provide higher pregnancy rates but require higher doses of gonadotrophins when compared with short protocols (Maheshwari et al., 2011). There is no evidence for a significant difference between depot and daily GnRH agonist using the long protocol but depot GnRH agonist requires more gonadotrophins and a longer duration of use and may therefore increase the overall costs of IVF treatment (Albuquerque et al., 2013). Single or multiple low-dose administration of GnRH antagonist during the late-follicular phase effectively prevents a premature rise in serum LH levels in most women (Tarlatzis et al., 2006). The use of antagonist compared with long GnRH agonist protocols is associated with a large reduction in OHSS with no evidence of a difference in live birth rates (Gilliam, 2011). Most recently, it has been implied that the use of GnRH antagonist may even hold out the prospect of running an IVF service that experiences no OHSS (Marci et al., 2013).

The full tool box

The availability of these new compounds will put new vigour into the methods used for ovarian stimulation... Each clinic will no doubt introduce its own protocols’ (Edwards et al., 1996).

The introduction of pharmaceutical grade recLH, complementing the earlier availability of recFSH (Devroye et al., 1992; Mannaerts et al., 1996), provided the long awaited opportunity to fine tune LH dose relative to FSH in ovarian stimulation regimens (Hiller, 2009). RecLH quickly proved helpful in designing safer and simpler protocols for stimulating monovulation in anovulatory women (Loumaye et al., 2003) but advantages for IVF ovulation induction are less evident (Mochtar et al., 2007; Durmerin et al., 2008). FSH alone—whether urinary or recombinant—is normally sufficient to yield a satisfactory ovarian response in long GnRH agonist IVF protocols (Marrs et al., 2004). RecFSH appears more effective than uFSH in inducing multifolliculation (Bergh et al., 1997; Out et al., 1997; Frydman et al., 2000; Schats et al., 2000), perhaps owing to its distinctive glycosylation pattern, which renders it more bioactive in vivo (Selman et al., 2013). RecFSH performs similarly (Hompes et al., 2008) or gives slightly lower live birth rates than HMG (Coomarasamy et al., 2008). LH supplementation does not improve ongoing pregnancy rates in women of 35 years or older (König et al., 2013) and confers no extra benefit in most other situations (Sills et al., 1999; Nardo et al., 2013).

The message is clear. Residual LH activity during pituitary suppression with a GnRH agonist is usually sufficient to support stimulation with exogenous FSH alone in most patients. Supplemental LH may yet prove beneficial in profoundly GnRH-agonist suppressed cycles (Balasch et al., 2003; Peñarrubia et al., 2003) or as ‘add-back’ in GnRH antagonist protocols (Garcia-Velasco et al., 2011; Griesinger and Shapiro, 2011).
Low-dose HCG has also been tried, as an alternative to recLH, to supplement FSH-containing gonadotrophins in IVF stimulation cycles (Filconi et al., 2005; Thuesen et al., 2012). HCG is essentially a long acting and more potent form of LH. Their molecular structures are similar but the HCGβ subunit has 24-amino acid C-terminal extension and is more heavily glycosylated than LHβ. This gives HCG a longer plasma half-life and higher bioactivity than LH in vivo (Alevizaki and Huhtaniemi, 2002).

An equivalent long-acting form of FSH has also been developed for clinical use, as a fusion product of FSH and the C-terminal peptide of the β-subunit of HCG. Its pharmacological activity is similar to normal FSH but with slower absorption rate and prolonged metabolic half-life (Croxtall and McKeage, 2011). A single SC dose of long-acting FSH is as effective as seven daily doses of recFSH in a standard GnRH antagonist protocol (Devroey et al., 2009; Powner et al., 2012).

Rec LH predictably affords an alternative ovulation trigger to HCG in IVF stimulation cycles. A single bolus of recLH is effective in inducing final follicular maturation and early luteinization in long GnRH agonist protocols and results in a highly significant reduction in OHSS compared with HCG. But, the dose of recLH required to achieve these effects is three to six times higher than HCG (European Recombinant LH Study Group, 2001; Emperaire and Edwards, 2004).

Rec HCG has also been evaluated against normal (i.e. urinary) HCG for triggering oocyte maturation in stimulated IVF cycles. Both perform similarly in terms of pregnancy rates and OHSS, therefore urinary HCG is still advocated for routine clinical use (Youssef et al., 2011a, b).

The inclusion of recombinant gonadotrophin preparations in the IVF pharmacopoeia has not changed the world, yet. However, as molecularly defined, pure drugs with proven safety and efficacy they open up new options for the future (Ludwig and Keck, 2005).

The full egg box

Methods to induce ovarian stimulation in amenorrhoeic and then in cyclic women 30 years ago have been greatly refined and extended in current practice. Curiously, a great many of them produce oocytes which fail to implant after fertilization, whether natural or induced cycles are being used. (Edwards, 2003)

The impetus to combine ovulation induction with IVF came from the expectation that more eggs and embryos for handling in vitro would enhance treatment outcome. Latterly, there was the added benefit that super-numerary eggs and embryos could be successfully cryostored to extend treatment options.

It rapidly emerged that IVF pregnancy rates were strongly influenced by the number of embryos replaced to the uterus. Multiple embryo transfer became the norm. Consequently, high-order multiple pregnancy was and remains one of the major risks of IVF therapy (Beall and DeCherney, 2012).

On average, one in five IVF pregnancies in the UK is a multiple pregnancy compared with 1 in 80 for women who conceive naturally. With ~13 000 IVF babies born each year, this substantially impacts the national multiple birth rate and creates a significant public health concern. Problems for the babies born include increased risks of stillbirth, neonatal death and disability. For mothers it increases the risk of dangerous complications to the mother, such as late miscarriage and pre-eclampsia (HFEA, 2013).

Scandinavian studies first demonstrated the clear benefits of elective single embryo transfer (eSET) to both mother and child after IVF (Hamberger et al., 2005). Subsequently, there has been a gradual shift towards limiting the number of oocytes (in the case of GIFT) or embryos replaced in order to minimize multiple pregnancy risk. In the UK, since 2009, the policy has been to encourage elective eSET and restrict multiple (double) embryo replacement to patients with poor prognosis based on age, number of previous failed attempts, etc. As the proportion of eSETs has increased, the multiple pregnancy rate has decreased (HFEA, 2013). The experience across Europe as a whole has been similar (Ferraretti et al., 2013). In this scenario, oocyte quality overrides oocyte quantity.

Back to the future

...endocrinological approaches, preferably reinforcing the natural cycle and acceptable for successive cycles of mild stimulation, are needed to-day. At most, modest forms of ovarian stimulation aimed at stimulating the growth of a limited number of Graafian follicles should suffice’. (Edwards et al., 1996)

Bob Edwards knew all along that the number of follicles ready to respond to FSH during any one cycle was limited. His best estimate was 5 or 6 per ovary (Edwards et al., 1972). In later years, he warned that high-order ovarian stimulation could be injurious to women’s health and called for a reassessment of the relevance on complex, costly treatment protocols resulting in large numbers of oocytes (Edwards et al., 1996; Edwards, 2007). He had in mind, gentler stimulation methods building on the natural cycle to yield fewer oocytes that more closely resemble the ‘gold-standard’ oocyte from a naturally ripened follicle (Fig 1).

The identity of the ‘gold-standard’ egg remains elusive (Li et al., 2008). Non-invasive techniques ranging from high-throughput screening of gene expression in cumulus cells (Assou et al., 2008) through genomic RNA profiling (Bell et al., 2008), metabolomics (Botros et al., 2008; Leese et al., 2008) and chromosomal assessment of the preimplantation embryo (Bell et al., 2008) have been brought to bear but none has been clinically validated to-date (Hillier, 2008).

Tactics exist for more subtle forms of stimulation based upon natural ovarian function (Hillier, 2009). No one size fits all. Personalized treatment based on antral follicle count and biomarkers of ovarian reserve such as anti-müllerian hormone (AMH) is gaining sway (Nelson, 2013). Natural cycle IVF has remained in the background since its successful introduction in 1978. Of the 47 003 fresh IVF cycles performed in the UK in 2011 using a woman’s own eggs, 596, 1.3%, were natural (Human Fertilisation and Embryology Authority, 2013). Minimal, mild or low stimulation protocols have also returned using FSH and/or clomiphene (Verberg et al., 2009; Zarek and Muasher, 2011). These upgraded regimens can now benefit from the optional addition of GnRH antagonist to prevent a natural LH surge and allow more convenient timing of egg collection to HCG. Variant GnRH agonist protocols are also available (Fauser et al., 2010). Maximizing protocols remain an option for women with reduced ovarian reserve.

Summary

The history of IVF endocrinology spans half a century. It began with Edwards’ assumption that hormone priming of the ovary would help treatment outcome. There was a lag phase when natural cycle IVF
IVF endocrinology

appeared to be the only way. Ovulation induction was gradually introduced, allowing the acceptance of IVF as a successful fertility treatment. Pragmatic and efficacy were substantially improved by programming with GnRH analogues. Powerful stimulation protocols geared to producing as many oocytes as possible became widespread. The whistles were blown (by Edwards). Attention returned to gentler forms of stimulation based on the natural ovarian cycle. Personalized treatment is now feasible, tailored to individual need. Yet, it remains to be seen if the relatively low cost, patient friendliness and safety of these protocols will mitigate the inherent fragility of working with reduced numbers of oocytes and embryos, which confounded the take up of natural cycle IVF in the first place.

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Authors’ roles

SGH conceived and executed the analysis upon which this review is based, and wrote the manuscript.

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