EDITORIAL

Minimal ovarian stimulation for IVF: appraisal of potential benefits and drawbacks

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Shortly after the first pregnancy following in-vitro fertilization (IVF) was reported in a spontaneous cycle, evidence accumulated that overall pregnancy chances per IVF attempt increase significantly when more than one embryo is transferred. Subsequently, ovarian stimulation protocols have been developed which aim at the ongoing growth of several follicles in normo-ovulatory women to obtain multiple oocytes for fertilization *in vitro* and multiple embryos. The presence of many pre-ovulatory follicles allows for easier oocyte retrieval, and less effective IVF, embryo culture and implantation.

Clearly, current ovarian stimulation protocols take many weeks, are extremely complex and expensive, and are not without danger. New procedures are frequently introduced without proper scientific evaluation (ISLAT working group, 1998) and current IVF strategies have been questioned (Edwards *et al.*, 1996; Olivennes and Frydman, 1998). In 1995, the worldwide number of IVF cycles was ~250 000 (de Mouzon and Lancaster, 1997). Overall birth rates have been reported at 6–25% per cycle. Problems related to stimulation include emotional stress, abdominal discomfort, risks of short-term complications and uncertainties regarding long-term health consequences for both the mother and children of multiple pregnancies. Some observations suggest that even singleton pregnancies resulting from IVF are more frequently complicated by bleeding, pre-eclampsia, diabetes or premature deliveries (Schenker and Ezra, 1994). Thus, the decision to undertake IVF treatment should not be considered lightly.

Approximately 25% of patients refrain from a second attempt after a first unsuccessful IVF cycle (P.Devroey, unpublished observations), even where the costs are reimbursed by insurance companies.

Ovarian hyperstimulation syndrome (OHSS) is a serious and potentially life-threatening complication of IVF characterized by enlarged ovaries, extravasation of fluid to the abdominal cavity resulting in ascites, hypovolaemia and haemoconcentration. Eventually, renal failure, thromboembolic complications and respiratory distress may occur. Severe forms of OHSS are associated with pregnancy, and related human chorionic gonadotrophin (HCG) production, which renders its management even more complex. The incidence of moderate OHSS is estimated to be ~6% per cycle, with severe cases approaching 2% (Aboulghar *et al.*, 1996; Elchalal and Schenken, 1997). This means an annual worldwide occurrence of at least 5000 cases of serious OHSS. Several patients are hospitalized every year in most major fertility centres. With careful monitoring of ovarian response, the risks of this serious complication can be reduced, but never brought to zero. The number of deaths resulting from OHSS is unknown, but with proper management the incidence should be low.

The health risks of hyperstimulation are easily quantifiable, but discussion continues about the other potential long-term health consequences of ovarian stimulation, especially concerning any association between stimulation and ovarian cancer. Although the epidemiological studies on gonadotrophin therapy published so far remain inconclusive (Shoham, 1994; Bristow and Karlan, 1996), it should be realized that experimental hypergonadotrophism and knock-out animal studies have suggested a relationship between long-term exposure to high gonadotrophin concentrations and the development of gonadal stromal tumours (Risma *et al.*, 1995; Kumar *et al.*, 1996; Kananen *et al.*, 1997). Although the situation in women has not yet been clarified, the risk of ovarian cancer is probably very low, if it exists. It should be realized that negative results are not usually published and that many previous reports were retrospective and poorly controlled. However, the reduced risk of ovarian cancer observed in steroid contraceptive pill users suggests a link between ovarian tumours and gonadotrophins (or ovulation).

It is also too early to exclude the possibility of risks, such as an earlier menopause due to increased follicular expenditure, although this seems highly unlikely because of the limited effects of follicle stimulating hormone (FSH) on a few follicles out of the thousands undergoing atresia. No evidence was obtained in studies on mice undergoing chronic treatment with pregnant mare's serum gonadotrophin (R.Gosden, unpublished observations). Finally, some studies have suggested that pro-

found ovarian stimulation impairs endometrial receptivity and subsequent rates of embryo implantation (Simón *et al.*, 1998).

Contemporary approach to presumed 'controlled' ovarian stimulation and embryo transfer for IVF

Over the years, the medication used for ovarian stimulation in IVF has included clomiphene citrate (CC), human menopausal gonadotrophin (HMG), and urinary FSH. Although still under investigation, it is increasingly clear that the overall IVF outcome has not improved significantly with the use of the recently introduced recombinant FSH preparations. Exogenous gonadotrophins are administered to stimulate multiple follicle development, usually at daily doses of two or three (but as high as six) ampoules, for 1–3 weeks, employing fixed, incremental or decremental protocols. A premature rise in serum luteinizing hormone (LH) concentrations due to early oestradiol positive feedback in relation to ovarian stimulation (believed to be detrimental to IVF outcome because of premature resumption of meiosis of the oocyte and a rise in progesterone) is prevented by the co-administration of gonadotrophin-releasing hormone (GnRH) agonists. This medication is usually initiated in the preceding cycle (so called 'long protocol') to induce complete pituitary down-regulation before the initiation of exogenous FSH. However, this medication increases the requirement for administered hormones because of suppressed endogenous gonadotrophins and may have an impact on subsequent ovarian responsiveness to exogenous FSH (Hugues and Cedrin-Durnerin, 1998). Resumption of oocyte meiotic maturation is induced by a single bolus injection of urinary HCG during the late follicular phase. HCG may also be used to support the corpus luteum, although exogenous progesterone supplementation is frequently applied in an attempt to avoid the risk of HCG stimulation of remaining growing follicles. Thus, the conventional IVF cycle is characterized by exogenous stimulation, often at high doses and abolition of physiological feedback factors.

It has become clear over the years that ovarian response to intense gonadotrophin stimulation is far from controlled (Baird and Pearson, 1998). Patient characteristics, rather than the stimulation protocol, seem to determine individual ovarian response. Ovarian ageing gives rise to a reduced number of growing follicles (hyporesponse) and significantly lower fertilization and implantation rates resulting in a greatly diminished IVF outcome (Templeton *et al.*, 1996). A reduced ovarian response cannot be overcome by changes in the stimulation protocol, e.g. by increasing the daily FSH dose (van Hooff *et al.*, 1993) or altering the timing and duration of stimulation (Rombauts *et al.*, 1998). It is crucial to be able to identify patients in advance that will elicit a poor response to standard stimulation. Inhibin B, produced by healthy early antral follicles (Groome *et al.*, 1996), may prove to be a suitable marker (Seifer *et al.*, 1997). The reason why some subjects hyperrespond to standard stimulation protocols remains unclear. So far, this condition cannot be predicted in most patients and represents a major risk of developing OHSS. A high rise in serum vascular endothelial growth factor during stimulation was recently shown to be a good predictor of hyperstimulation

(Agarwal *et al.*, 1999). Patients suffering from polycystic ovary syndrome are known to be at risk (MacDougall *et al.*, 1993), but the pharmacokinetics of FSH are also unpredictable, presumably because of inter- and intra-individual differences in gonadotrophin absorption and clearance (Ben Rafael *et al.*, 1986). The administration of gonadotrophins in decremental doses may reduce ovarian response in these patients (Simón *et al.*, 1998).

No agreement exists regarding the optimal number of oocytes required for IVF. Individual responses to standard treatment vary greatly, but in most cases 8–15 oocytes are obtained. The question as to whether stimulation of large numbers of Graafian follicles inversely affects oocyte quality remains unanswered. Fertilization rates of retrieved oocytes are ~70% *in vitro*. The transfer of more than one embryo results in higher overall pregnancy rates, but clearly at the cost of an increased incidence of (higher order) multiple pregnancies. Overall multiple pregnancy rates of 27% with 4% triplets (equivalent to 60 triplets per year) occurred in France from 1986 to 1990 (FIVNAT, 1995, 1996). In the USA and Canada, the birth rate of multiple pregnancies was even higher at 39% in 1996, with 856 triplet (accounting for 5.8% of all deliveries) and 71 quadruplet (0.5%) deliveries (Society for Assisted Reproductive Technology, 1999). In 1995 in the USA, the average number of embryos transferred was still four, with triplets or higher order births at 2.6% per embryo transfer (Center for Disease Control and Prevention, 1998). The frequency of multiples clearly reflects the number of embryos transferred. It remains unknown how many higher order multiple pregnancies are selectively reduced or abort spontaneously. Hence, the percentage of multiple pregnancies generated through IVF should be substantially higher. In the USA, it was recently proposed to set a maximum number of four to six embryos for transfer, depending on the age of the woman (Muasher, 1998). There is no consensus over what extent large scale fetal reduction is ethically acceptable. The attitude of aiming for high overall pregnancy rates regardless of the incidence of multiples may be stimulated by the introduction of 'money-back guarantees' for fertility services (Andreck *et al.*, 1998).

Although emphasized in recent literature (Callahan *et al.*, 1994), obstetric complications, fetal morbidity and mortality, costs and the psychosocial consequences of multiple pregnancies are still insufficiently appreciated by physicians working in infertility clinics. In a managed care setting, the resources consumed by a multiple gestation run to several millions of $US per year due to long-term hospitalization, Caesarian section, and intensive neonatal care. Despite all these efforts, human suffering due to neonatal morbidity and mortality remains high. Such excessive consumption of resources will preclude other therapies and will draw restriction responses if not addressed by the practitioners of infertility care. Avoidance of multiple pregnancies rather than fetal reduction should be the aim. Efforts are being made to reduce the incidence of (higher order) multiple gestation by limiting the number of embryos transferred (Bronson, 1997). A recent retrospective analysis of a large database from the UK suggested that the number of multiples could be diminished without an overall

decrease in the births rate, when the number of embryos transferred was reduced from three to two (Templeton and Morris, 1998). However, even in the more restrictive European IVF centres, approximately half of all IVF children born today are from multiple pregnancies.

Cryopreservation of supernumerary embryos for transfer in subsequent (unstimulated) cycles is often used to justify the stimulation of large numbers of follicles, though the maximum birth rates obtained by this strategy are only 8% (Mandelbaum et al., 1998). Nevertheless, there is the advantage that repeated ovarian stimulation and painful oocyte retrieval procedures can sometimes be avoided by the transfer of cryopreserved embryos. Although hundreds of pregnancies have been reported, the real benefits of cryopreservation programmes are still under debate (Jones et al., 1997) and the increase in patient-specific pregnancy rates (i.e. increased chance of an ongoing pregnancy from a cryotransfer after a failed fresh transfer) appears substantially less than generally perceived. In addition, legal and ethical issues (as well as long-term safety) related to cryopreserved embryos have not been resolved. The possibility of cryopreserving supernumerary oocytes rather than embryos has recently been proposed (Porcu et al., 1998, 1999) and the results of clinical trials are watched with great interest.

Follicle growth and single dominant follicle selection during the normal menstrual cycle

More focus on ovarian physiology is required for the design of new treatment strategies (Hillier et al., 1985; Oehninger and Hodgen, 1990). Development of a follicle from the primordial up to the pre-ovulatory stage takes several months. Initiation of the growth of primordial follicles occurs continuously and in a random fashion (Gougeon, 1996). It remains to be clarified how early follicle development and atresia are regulated independently from gonadotrophins. New factors affecting follicle development, e.g. growth differentiation factor-9 (Dong et al., 1996), Bax (Perez et al., 1999), Wilms tumour 1, C-*kit* and other factors regulating apoptosis (i.e. programmed cell death) (Hsueh et al., 1996) are coming to light. The role of FSH at early stages of follicle development is still under investigation (Oktay et al., 1997), but seems limited. Healthy, early antral follicles measuring 2–5 mm in diameter are present throughout the entire menstrual cycle (McNatty et al., 1983; Chikazawa et al., 1986). These follicles will only continue to grow when sufficiently stimulated by the rise in serum FSH concentrations at the beginning of the cycle (when the corpus luteum involutes) (Hodgen, 1982; Baird, 1990; Zeleznik, 1993). The cohort size of the healthy early antral follicles recruited per cycle is believed to be ~10–20 in young women (Hodgen 1982; Pache et al., 1990; Gougeon, 1996).

A decline in FSH during the follicular phase appears to be crucial for selection of a single dominant follicle (van Santbrink et al., 1995), i.e. the one escaping atresia by becoming more sensitive to stimulation by FSH (Fauser and van Heusden, 1997). The dominant follicle can be distinguished from other cohort follicles by its larger size (>10 mm diameter), higher oestradiol/androgen ratio (van Dessel et al., 1996), the acquisition of LH responsiveness and greater vascular supply. Other cohort follicles can be rescued from atresia and stimulated to develop as dominant follicles by the administration of low doses of exogenous FSH during the mid- to late follicular phase, effectively preventing a decrease in serum FSH (Schipper et al., 1998; De Jong et al., 1999).

Natural cycle and minimal ovarian stimulation for IVF

In sharp contrast to the standard method of intense ovarian stimulation, attempts to improve the outcome of IVF during the spontaneous menstrual cycle have continued over the years. Results in several hundreds of patients have been reported with cancellation rates of 10–30%, single egg recovery rates of 75–90%, fertilization rates of 60–80%, implantation rates per single embryo transfer of 20–30%, and overall ongoing pregnancy rates per started cycle from 5–15% (Foulot et al., 1989; Lenton et al., 1992; Seibel, 1994; Society for Assisted Reproductive Technology, 1996). Clearly, this approach is less invasive and cheaper, but it is also less effective. Cancellation rates remain high and unpredictability of the timing of the LH surge imposes logistic difficulties. The occurrence of an LH surge may be prevented by the administration of a GnRH antagonist in the late follicular phase (Olivennes and Frydman, 1998; Rongieres-Bertrand et al., 1999) which reduces cancellation rates and may render this approach more flexible.

The clinical introduction of GnRH antagonists provides novel opportunities to review current strategies for profound ovarian stimulation in IVF. GnRH antagonist action is characterized by an immediate suppression of pituitary gonadotrophin release and a rapid recovery of normal secretion of endogenous LH and FSH (Hall, 1993). The mid-cycle LH surge requires the presence of endogenous GnRH and can, therefore, be prevented or inhibited by the administration of GnRH antagonist. Recent studies have shown that GnRH antagonists (either at single or multiple doses) are effective in preventing a premature LH rise during ovarian stimulation for IVF (Frydman et al., 1991; Diedrich et al., 1994; Olivennes et al., 1995; Diedrich and Felberbaum, 1998) and its clinical efficacy has been confirmed by large multi-centre phase III clinical trials. However, these studies seem to suggest that implantation rates per transferred embryo are reduced in GnRH antagonist-stimulated cycles.

Thus, a treatment cycle can commence with an undisturbed menstrual cycle and recruitment of a normal cohort of about 10 healthy, early antral follicles. Exogenous FSH administered during the mid- to late follicular phase prolongs growth of some of these follicles up to the pre-ovulatory stage (De Jong et al., 1999). By making optimal use of endogenous FSH, the amount of exogenous FSH required should be substantially reduced. Additional studies are required to establish the optimal starting day and dose of exogenous FSH, as well as the number and quality of oocytes obtained (Table I).

Implications and future development of minimal stimulation protocols

Firstly, the application of minimal stimulation protocols will result in a reduced duration of stimulation and lower amounts

Table I. Minimal ovarian stimulation with gonadotrophin-releasing hormone (GnRH) antagonist co-treatment for in-vitro fertilization (IVF): potential advantages, disadvantages and outstanding questions

Advantages	Disadvantages	Questions for investigation
Less patient discomfort	fewer oocytes for fertilization *in vitro*	overall live birth rates
Less complex, shorter stimulation regimens	IVF becomes less programmable	effects on oocyte quality and fertilization rates
Less chances of short-term complications	fewer spare embryos for cryopreservation	effects on embryo quality
Less chances of long-term health risks		effects on endometrial receptivity
Less expensive		luteal support requirement

of exogenous FSH. Less monitoring will be required and the chances of short-term complications and long-term risks are expected to be reduced. Drawbacks to this approach include the fact that IVF procedures will become less programmable, because each IVF cycle will start with the onset of spontaneous menses rather than long-term suppression by GnRH agonists which allows the initiation of stimulation to be chosen. However, timing may be more controllable by the use of oestrogen preparations alone or in combination with gestagens in the late luteal phase to postpone menses (de Ziegler *et al.*, 1998). Minimal stimulation may prove to be less effective in women of advanced reproductive age and the overall effectiveness of minimal stimulation protocols remains to be established in large series of patients.

Improved laboratory techniques for oocyte maturation *in vitro* rather than *in vivo* may further reduce, or even abandon, the need for exogenous gonadotrophin stimulation in the future (Barnes *et al.*, 1996; Oktay *et al.*, 1998). To date, in the hands of some investigators, ~80% of oocytes obtained from medium sized antral follicles mature to metaphase II, and ~85% of these oocytes fertilize and cleave *in vitro* (Cha and Chian, 1998). However, embryo quality seems to be compromised and only a few pregnancies have been reported so far. In the long-term, it should be possible to recover fertilizable oocytes grown *in vitro* from primordial or primary follicles, and after frozen banking if required.

Secondly, the strategy of administering low doses of FSH in the late follicular phase will result in fewer oocytes being available for IVF than in conventional stimulation protocols. It may also be possible to selectively stimulate the growth of large follicles in the late follicular phase by the administration of low doses of LH. Recent observations indicate that once FSH has initiated follicular growth, thereafter either FSH or LH is capable of sustaining follicular oestradiol production by acquired LH responsiveness of the dominant follicle (Sullivan *et al.*, 1999).

The smaller cohort of growing follicles may well be better synchronized which may improve oocyte quality and the capacity to be fertilized and form good quality embryos. Reduced stimulation may also avoid the adverse effects of hyperstimulation on corpus luteum function and endometrial receptivity.

Thirdly, with the use of GnRH antagonists during the late follicular phase, the final stages of oocyte meiotic maturation can also be induced by the administration of recombinant LH, recombinant FSH, native GnRH or GnRH agonist instead of HCG (Olivennes *et al.*, 1996). These approaches are currently under investigation and may further reduce the risk of OHSS.

Effects on oocyte quality and subsequent IVF outcome of these alternative stimuli have not yet been established in clinical trials.

It has been clearly established that corpus luteum support is required following ovarian stimulation combined with GnRH agonist (Smitz *et al.*, 1987; Belaisch-Allart *et al.*, 1990; Akande *et al.*, 1996). This may be due to prolonged suppression of pituitary function following GnRH agonist cessation, since LH is luteotrophic. However, other causes of abnormal pituitary function during the luteal phase in IVF cycles should also be considered, such as an effect of ovarian stimulation *per se*, administration of large quantities of HCG in the late follicular phase, puncture of follicles and removal of large quantities of granulosa cells or augmented feedback by distinctly elevated luteal phase oestradiol and progesterone concentrations. A rapid recovery of pituitary LH and FSH release after cessation of GnRH antagonist administration might permit the abandonment of additional luteal support and the further simplification of IVF protocols.

Fourthly, milder forms of ovarian stimulation for IVF are likely to generate fewer embryos. The transfer of large numbers of embryos to increase 'success' rates per IVF cycle or to compensate for poor laboratory performance can no longer be justified. Higher order multiple pregnancies should not be considered an IVF success. The aim should be to reduce the risks of multiple pregnancies rather than refining techniques for fetal reduction.

The choice of embryo to transfer on the basis of morphology assessment at the 4- or 8-cell stage (i.e. cell number, their regularity and degree of fragmentation) seems to be rather unreliable. When implantation rates per embryo can be improved by prolonged culture for five days and transfer of one or two rapidly developing viable embryos at the blastocyst stage (Gardner and Lane, 1997), the incidence of multiple pregnancies may be further reduced without a fall in overall pregnancy rates (Scholtes and Zeilmaker, 1996; Gardner *et al.*, 1998; Vilska *et al.*, 1998; Wolner-Hanssen and Rydhstroem, 1998). A new generation of culture media is enabling up to 50% of embryos to reach the blastocyst stage, and implantation rates per embryo of 30–50% have been reported. Much may be gained by further improving embryo culture conditions and identifying genetically competent good quality embryos, preferably using non-invasive methods. Embryology is crucial if IVF success rates are to improve much further.

Fifthly, milder stimulation protocols may result in fewer or no spare embryos being available for freezing. However, while the transfer of cryopreserved embryos in subsequent cycles represents additional chances for pregnancy without the need

for ovarian stimulation and oocyte retrieval, the true added value of cryopreservation programmes remains to be established. The effectiveness of cryopreservation may also depend on the developmental stage of the embryo.

Sixthly, the duration of an IVF cycle will be significantly reduced with minimal stimulation combined with GnRH antagonist. Moreover, this type of stimulation will be much better tolerated by the patient. Therefore, more IVF attempts may be performed in a given period of time with less risks and at similar costs. Further studies should be undertaken to substantiate this concept.

Advances in reproductive technology should be viewed as a multi-disciplinary area of clinical science, as in other medical fields. Much of the development in IVF worldwide has been in the private sector, an environment not usually focused on well-conducted clinical trials. Moreover, the field is heavily dominated by pharmaceutical companies. Finally, little research money is available for clinical reproductive medicine. This may be responsible for the early implementation of poorly validated strategies.

Only after the above-mentioned approaches have been validated will the balance of advantages be apparent between complexity, duration of treatment, patient discomfort, risks and complications, costs, and efficiency in producing a healthy singleton pregnancy. Less complex ovarian stimulation protocols, together with improved embryo selection and endometrial receptivity are of crucial significance for the further advancement of IVF. It seems likely to us that the current paradigm of intense ovarian stimulation protocols for IVF will be abandoned early in the next millennium when new medication and more refined laboratory techniques become available. Efforts in clinical research in this area, unrestricted by the interest of commercial sponsors, needs to increase dramatically. This will be an investment for society in general and infertility patients specifically, that returns itself in both the quality of outcomes as well as several readily quantifiable measures of health care outcomes.

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