

Regulation of follicle development and novel approaches to ovarian stimulation for IVF

N.S.Macklon* and B.C.J.M.Fauser

Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands

Current ovarian stimulation regimens for IVF are complex and not without risk. Increasing our knowledge of the physiology of follicle development and dominant follicle selection may enable the design of less complex, safer and cheaper ovarian stimulation regimens for IVF. Decremental serum FSH concentrations during the follicular phase of the menstrual cycle are required for single dominant follicle selection. Only the most mature follicle will continue its development due to increased sensitivity for stimulation by FSH. FSH stimulation becomes insufficient for less mature follicles and remaining cohort follicles will therefore go into atresia. The number of days during which FSH is above the threshold for stimulation of follicle development is limited, resulting in a narrow FSH window. More medium sized and large pre-ovulatory follicles and increased oestradiol output can be induced by the administration of small doses of exogenous FSH during the mid- to late follicular phase, preventing the physiological decrease in FSH stimulation. Intervention with decremental serum FSH concentrations in combination with gonadotrophin-releasing hormone (GnRH) antagonists to prevent a premature rise in serum LH may induce ongoing growth of multiple follicles sufficient for IVF. The benefits and risks of these minimal hyperstimulation protocols require further evaluation.

Key words: follicle development/GnRH antagonists/IVF/ovarian stimulation

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Mechanisms of single dominant follicle selection during the normal menstrual cycle

Initiation of growth of primordial follicles occurs continuously and in a random fashion (Gougeon, 1996) and development from the primordial up to the pre-ovulatory stage takes several months. The regulation of early follicle development and atresia and the degree to which early stages of follicle development are influenced by FSH remains unclear (Oktay *et al.*, 1997), but recent evidence suggests that factors regulating apoptosis (i.e. programmed cell death) are involved (Hsueh *et al.*, 1996). Morphological and endocrine studies indicate that antral follicles measuring <4 mm in diameter are present throughout the cycle

and are continuously available for stimulation by FSH. At the end of the luteal phase, the largest healthy follicles observed by morphological criteria are 2–5 mm in diameter (McNatty *et al.*, 1983; Chikazawa *et al.*, 1986; Gougeon, 1986). Granulosa cells obtained from follicles in the late luteal phase are more sensitive to FSH stimulation (McNatty *et al.*, 1983) suggesting that these follicles will be recruited for the next cycle. Indeed, these follicles will continue to grow when sufficiently stimulated by rising FSH concentrations (Hodgen, 1982; Baird, 1990; Zeleznik, 1993). Due to demise of the corpus luteum and subsequent decreased oestrogen output (le Nestour *et al.*, 1993), FSH concentrations rise at the end of the luteal phase (Hall *et al.*, 1992). During the subsequent follicular phase, FSH concentrations plateau during initial days (van Santbrink *et al.*, 1995) and are gradually suppressed thereafter by ovarian steroid and inhibin negative feedback. Although each growing follicle may initially have an equal potential to reach full maturation, only those follicles that happen to be at a more advanced stage of maturation during this inter-cycle rise in FSH (concentrations surpassing the so called threshold for ovarian stimulation) gain gonadotrophin dependence and continue to grow. Based on indirect observations it is believed that the cohort size of healthy early antral follicles recruited during the luteo-follicular transition is ~5–10 per ovary (Hodgen, 1982; Pache *et al.*, 1990; Gougeon, 1996).

*To whom correspondence should be addressed at: Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. Phone: ; Fax 0031 10 436 7306; E mail: macklon@gyna.azr.nl

Decremental follicular phase FSH concentrations (effectively restricting the period for which FSH concentrations remain above the threshold, referred to as the FSH window) appear to be crucial for selection of a single dominant follicle from the recruited cohort (van Santbrink *et al.*, 1995). Only one follicle escapes from atresia by increased sensitivity for stimulation by FSH (Fauser and van Heusden, 1997). Many factors are involved in the reduced requirement for FSH stimulation of the dominant follicle, including an increased number of granulosa cells, the acquisition of LH receptors and increase in FSH receptors on granulosa cells, combined with autocrine and paracrine up-regulation by multiple intra-ovarian growth factor systems. In-vitro studies have suggested these include insulin-like growth factors (IGF), transforming growth factor- β , fibroblast growth factor and activin, while showing other growth factors, e.g. inhibin, epidermal growth factor and IGF binding proteins to have an inhibitory effect on FSH-stimulated granulosa cell function *in vitro*. In-vitro studies have also shown intrafollicular changes in the IGF system to be independent of those observed in the serum (Van Dessel *et al.*, 1999). Cytokines, e.g. tumour necrosis factor- α and interleukins 1, 2 and 6 have also been shown *in vitro* to have an inhibitory effect on gonadotrophin-induced steroidogenesis. Overall, the true physiological significance of these factors remains uncertain however, as human *in-vivo* studies have been unable to clearly confirm their individual role. An alternative mechanism by which the reduced requirement for FSH by the dominant follicle might be explained has been proposed on the basis that different isoforms of FSH, each demonstrating differing degrees of biopotency, predominate during each stage of the follicular phase. The predominance of more biopotent, shorter half-life basic isoforms of FSH in the late follicular phase supports a role for changing FSH isoform profile in dominant follicle selection. However, data from *in-vivo* human studies is lacking and the true physiological significance of this observation remains open to speculation (Fauser and van Heusden, 1997). The important concept of increased sensitivity of the dominant follicle for FSH has been confirmed by human studies showing developing follicles to exhibit a variable tolerance for GnRH antagonists-induced gonadotrophin withdrawal (Fluker *et al.*, 1991; Hall *et al.*, 1991).

The dominant follicle can be distinguished from other cohort follicles by size (>10 mm diameter) (Pache *et al.*, 1990; van Santbrink *et al.*, 1995), morphology (Bomsel-Helmreich *et al.*, 1979), intrafollicular endocrine changes (van Dessel *et al.*, 1996) and by demonstration of induction of aromatase enzyme activity (Erickson and Yen, 1984). These studies have shown that enhanced oestradiol biosynthesis is closely linked to pre-ovulatory follicle development. Ultrasound studies of follicular development have confirmed this morphological-endocrine link. The first ultrasound demonstration of this was published by Hackeloer *et al.* (1977) using trans-abdominal techniques, and subsequent studies confirmed a close correlation in individual patients between follicle size and serum oestrogen concentrations (Kerin *et al.*, 1981). Transvaginal ultrasound, available since 1985, has enabled more reliable assessment of changes in size and number of follicles. In studies of follicular-endocrine dynamics in the normal cycle, our group has characterized the decremental follicular phase patterns of FSH serum concentrations (Schipper *et al.*, 1998a) and investigated the correlation between the

decreasing FSH concentrations and dominant follicle development (van Santbrink *et al.*, 1995). This decrease in FSH concentrations may be due to negative oestrogen feedback on the hypothalamic axis (Baird, 1987). A profound rise in inhibin B serum concentrations was recently observed in the follicular phase (Groome *et al.*, 1996) suggesting that it is secreted by recently recruited cohort follicles in response to FSH. This rapid rise in inhibin B occurs just after the intercycle rise in FSH. Inhibin B may therefore limit the duration of the FSH rise (narrowing the FSH 'window') through negative feedback at the pituitary and could therefore be crucial for monofollicular development.

The administration of low doses exogenous FSH during the mid follicular phase can disrupt these mechanisms for single dominant follicle selection by effectively preventing a decrease in serum FSH and therefore widening the FSH window (Schipper *et al.*, 1998b). Moreover, when normally ovulating volunteers are given exogenous FSH as late as cycle day 7, follicles from the recruited cohort can be rescued from atresia and stimulated to dominance (Hohmann *et al.*, 2000). While the precise moment of dominant selection remains uncertain and may vary from woman to woman and from cycle to cycle, these findings taken together suggest that selection of the dominant follicle occurs relatively late during follicle development, after the recruitment of a pool of follicles. The observation that healthy follicles at the start of the follicular phase vary in size of 4–8 mm but show no morphological differences between them (Gougeon and Lefevre, 1983) supports the concept of late selection to dominance. In our studies of 40 normally menstruating volunteers who underwent daily transvaginal ovarian ultrasound, we found the dominant follicle to be identifiable from median cycle day 7 (Figure 1). The normal endocrinology of the ovulatory menstrual cycle in these women and the pattern of growth of the dominant follicle from day of dominance up to the day on which the LH peak occurs are shown in Figure 2. During the seven days prior to the LH peak, the diameter of the dominant follicle increased linearly by mean value of 1.1 mm/day reaching a median pre-ovulatory size of 20.5 mm (Figure 3). Pre-ovulatory serum and follicular fluid oestradiol concentrations correlate significantly with the size of the pre-ovulatory follicle (Figure 4).

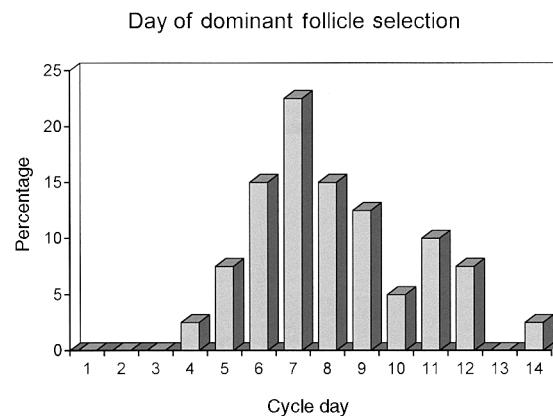


Figure 1. The day on which the dominant follicle was first identified in normally cycling women ($n = 40$) who underwent daily ultrasound examinations is shown, expressed as a percentage of the study group examined.

Impact of current paradigms of controlled ovarian stimulation for IVF

Ovarian stimulation protocols applied worldwide today take several weeks per stimulated cycle, are complex, expensive, and associated with a certain degree of risk (Fauser *et al.*, 1999). Gonadotrophin preparations are administered to stimulate multiple follicle development usually at daily doses of two or three (but sometimes more than six) ampoules for 1–3 weeks. A premature rise in serum LH concentrations, generally believed to be detrimental for IVF outcome, is prevented by the co-

administration of a gonadotrophin-releasing hormone (GnRH) agonist which is usually initiated in the preceding cycle to allow for pituitary down-regulation to occur before the initiation of exogenous FSH. Finally, resumption of oocyte meiotic maturation is induced by a single bolus injection of human chorionic gonadotrophin (HCG) during the late follicular phase. In addition, the corpus luteum is supported during the luteal phase by repeated administration of HCG or supplemented by exogenous progesterone.

It has become clear that ovarian response to such intense gonadotrophin stimulation is far from controlled. Indeed, undergoing such treatment regimens imposes both emotional and physical burdens on the patient while exposing her to the risk of both short-term complications and possibly long-term health consequences. Short-term risks involve ovarian hyperstimulation syndrome (OHSS). The availability of multiple embryos may, in certain clinics, be exploited by means of multiple transfer to increase pregnancy rates at the expense of an increased incidence of (higher order) multiple pregnancies. OHSS is characterized by enlarged ovaries, extravasation of fluid to the abdominal cavity resulting in ascites, hypovolaemia and haemoconcentration and eventually renal failure, thromboembolic complications and respiratory distress. The incidence of severe OHSS is ~2% (Aboulghar *et al.*, 1996; Elchalal and Schenken, 1997) and several patients are hospitalized per year for OHSS in every major fertility centre. Some women may die from complications, e.g. kidney failure. With the use of recombinant (r)FSH, serum oestradiol assays have become less reliable in predicting OHSS (Fauser, 1997a) and assessment of number and size of follicles by transvaginal ultrasound constitutes the primary monitoring tool. Although the underlying mechanism of OHSS is still unclear, recent studies suggest that a local ovarian renin angiotensin system and vascular endothelial growth factor (VEGF) may play a

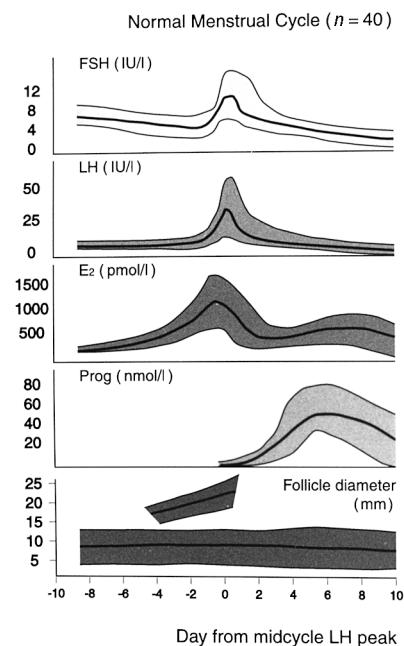


Figure 2. Mean serum concentrations with 95 percentiles are given for LH, FSH, oestradiol and progesterone from 8 days prior to the mid-cycle LH peak until 10 days thereafter. The bottom panel shows the emergence and growth of the dominant follicle in relation to the time of the mid-cycle LH peak. Data obtained from regularly cycling women ($n = 40$).

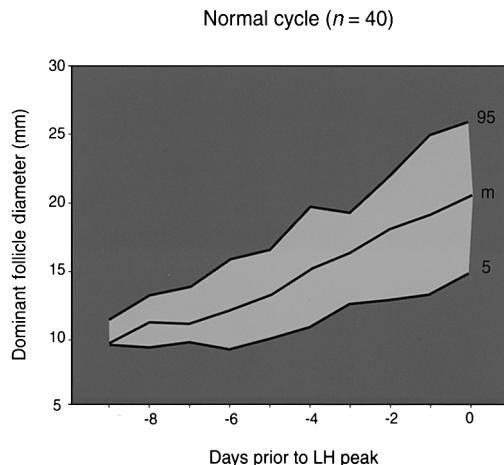


Figure 3. The mean diameter of the dominant follicle measured ultrasonically in normally cycling women ($n = 40$) on the day of the LH surge, and the 9 days prior to this is shown. The inner line indicates the mean diameter values, while the outer lines show the 5 and 95% confidence intervals.

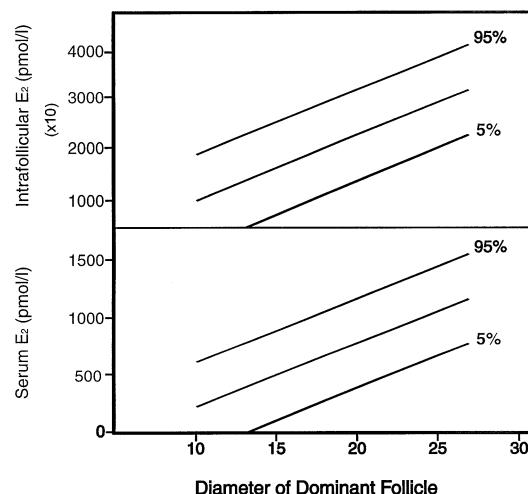


Figure 4. Upper panel: The regression line with 5 and 95% confidence intervals for serum oestradiol (E₂) concentrations during development of the dominant follicle is shown for normally menstruating women ($n = 40$). Lower panel: The regression line with 5 and 95% confidence intervals for follicular oestradiol concentrations during development of the dominant follicle is shown for normally menstruating women ($n = 55$) (data taken from Van Dessel *et al.*, 1996).

major role in the pathogenesis of OHSS through an effect on angiogenesis and capillary permeability (Ong *et al.*, 1991; McClure *et al.*, 1994). The prevention of severe OHSS remains primarily dependent on the avoidance of pregnancy in women at risk as a result of undergoing an IVF stimulation cycle. Cryopreservation and interval transfer during a natural or induced ovulatory cycle may be an alternative option in these cases. Other approaches to the prevention and management of OHSS include the administration of i.v. albumin. Although three randomized controlled studies suggest a prophylactic effect (Shoham *et al.*, 1994; Shalev *et al.*, 1995; Isik *et al.*, 1996), different inclusion criteria were used, and the true efficacy of i.v. albumin in this clinical context remains uncertain.

With regard to long-term health risks, an association between ovarian stimulation and ovarian cancer has been proposed for many years but epidemiological studies and case reports published so far remain inconclusive (Shoham, 1994; Tarlatzis *et al.*, 1995; Bristow and Karlan, 1996) and large prospective epidemiological studies will be required to clarify this issue. In the meantime it would appear prudent to limit exposure to gonadotrophins and their effects on the ovary to the minimum required for successful fertility treatment.

Finally, the potentially detrimental effects of profound ovarian hyperstimulation on endometrial receptivity and subsequent embryo implantation rates should be appreciated (Simón *et al.*, 1995).

Although there is no agreement regarding the optimal number of oocytes required, current strategies for ovarian stimulation in IVF aim to produce 10–20 oocytes (Fauser *et al.*, 1999). The question as to whether stimulation of large numbers of Graafian follicles inversely affects oocyte quality remains open for speculation. Fertilization rates of mature oocytes *in vitro* are >70% if sperm quality is sufficient (Fauser *et al.*, 1999). When sperm quality is poor, similar fertilization rates can be reached with the use of intracytoplasmic sperm injection. The transfer of more than one embryo results in higher overall pregnancy rates, at the cost of an increased incidence of (higher order) multiple pregnancies. Even today, many clinics throughout the world transfer four or more embryos. Overall multiple pregnancy rates of 25% with 4% triplets have been reported recently in France (FIVNAT, 1995, 1996), and 36% multiple pregnancies in the USA and Canada (Society for Assisted Reproductive Technologies/SART, 1999). Although emphasized in recent literature, obstetric complications, fetal morbidity and mortality, costs and psychosocial consequences of multiple pregnancies are still insufficiently appreciated by physicians working in infertility clinics. In Europe, efforts are being made to reduce the incidence of (higher order) multiple gestation by limiting the number of embryos transferred (Bronson, 1997).

Cryopreservation of supernumerary embryos and transfer in subsequent (unstimulated) cycles is often considered to justify the stimulation of large numbers of follicles. However, the true added value of cryopreservation programmes is still under debate (Jones *et al.*, 1997) and the resulting increase of patient specific pregnancy rates (i.e. increased chance of an ongoing pregnancy from a frozen transfer after a failed fresh transfer) may be less than generally perceived. In view of the many

legal and ethical issues relating to cryopreserved embryos, the possibility of cryopreserving supernumerary oocytes rather than embryos has recently been proposed (Porcu *et al.*, 1997).

Given the above concerns, it is unsurprising that current strategies of ovarian stimulation for IVF are increasingly being questioned (Edwards *et al.*, 1996; Olivennes and Frydman, 1998; Fauser *et al.*, 1999). In order to design new treatment strategies aimed at producing a limited but sufficient number of Graafian follicles for IVF, more focus on ovarian physiology is required (Hillier *et al.*, 1985; Oehninger and Hodgen, 1990).

Minimal ovarian stimulation for IVF

The clinical introduction of GnRH antagonists has made feasible the development of new paradigms for ovarian stimulation in IVF (Bouchard and Fauser, 2000). 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 secretion of native GnRH and can therefore also be prevented or inhibited by GnRH antagonist administration. Recent studies have shown that GnRH antagonist, either at single or multiple doses, is effective in suppressing a premature LH surge during ovarian stimulation for IVF (Frydman *et al.*, 1991; Diedrich *et al.*, 1994; Olivennes *et al.*, 1995; Albano *et al.*, 1996) and its clinical efficacy is presently being confirmed in large multi-centre phase 3 clinical trials (unpublished observations). This novel approach in IVF allows for the initiation of a normal menstrual cycle with normal early follicular phase recruitment of a cohort of follicles.

When, in women of normal reproductive age, ~10 healthy early antral follicles are stimulated to ongoing growth by the endogenous inter-cycle rise in FSH, the aim of exogenously administered FSH may merely be to stimulate growth of these follicles up to the pre-ovulatory stage (De Jong *et al.*, 2000). By making optimal use of endogenous FSH, the amount of FSH administered for an adequate response should be substantially less. Additional studies should establish the optimal starting day and dose of exogenous FSH required to prevent the decrease in serum FSH concentrations responsible for single dominant follicle selection under normal conditions. So far, studies with GnRH antagonists all involve the initiation of FSH administration on cycle days 2 or 3. However, maximum endogenous FSH concentrations are reached on median cycle day 6, and dominance is achieved on cycle day 7 in normo-ovulatory women (van Santbrink *et al.*, 1995; Schipper *et al.*, 1998a). Indeed, preliminary observations suggest that multiple follicle development and IVF pregnancies can be achieved with daily doses as low as 100 IU rFSH starting on cycle day 5 (De Jong *et al.*, 2000). This concept is currently under investigation in a larger, comparative clinical trial.

With the use of GnRH antagonists during the late follicular phase, final stages of oocyte meiotic maturation can also be induced by the administration of rLH, rFSH, native GnRH or GnRH agonist instead of HCG (Olivennes *et al.*, 1996). These approaches are currently under investigation and may also reduce the risk of OHSS (De Jong *et al.*, 1998).

Future directions

The impact of recombinant gonadotrophins and GnRH antagonists on IVF practice is now becoming discernable. However, potentially exciting developments also lie in the modification of recombinant gonadotrophins (in particular FSH) to manipulate their biopotency and half-life. By altering the glycosylation of FSH, it may be possible to develop short-acting but potent rFSH fractions (basic FSH) and long-acting less potent fractions (acidic FSH) (Fauser, 1997b). Adding the carboxy-terminal peptide (CTP) of the HCG β subunit (known to be responsible for the long half-life of the compound) to FSH β has been shown to allow extension of the half-life of the resultant compound, FSH-CTP. This has similar receptor binding and in-vitro bioactivity to rFSH, but enhanced circulating half-life and in-vivo biopotency (La Polt *et al.*, 1992). Clinical trials of FSH-CTP are now underway and may lead to further simplification and economy in ovarian stimulation for IVF.

Conclusions

The approach of interfering with single dominant follicle selection by late follicular phase administration of low doses of FSH may result in a reduced number of oocytes available for IVF as compared with currently applied stimulation protocols. Further studies are required to establish convincingly whether oocyte quality and subsequent capacity to be fertilized and form good quality embryos is improved when fewer follicles are stimulated. This could potentially represent an advantage over milder forms of ovarian stimulation. In addition, discussion continues as to whether profound ovarian stimulation *per se* may have a negative impact on corpus luteum function and on endometrial receptivity. A recent study suggested that the implantation rate per embryo is affected by the magnitude of follicular phase ovarian hyperstimulation, independent from embryo quality (Simón *et al.*, 1995). The administration of gonadotrophins in decremental doses in patients with a previous hyper-response to standard stimulation regimens may reduce ovarian response in these patients and improved implantation rates (Simón *et al.*, 1998).

The application of minimal stimulation protocols will result in a significantly reduced duration of stimulation and significantly lower amounts of exogenous FSH preparations needed. Less monitoring will be required and chances for short-term complications or long term risks may be reduced. This approach is likely to render IVF more cost-effective. Drawbacks of this approach include the fact that IVF procedures will become less programmable, because an IVF cycle starts with the onset of spontaneous menses rather than long-term suppression of endogenous gonadotrophins by GnRH agonists where initiation of stimulation can be chosen. However, timing may be improved by the use of oestrogen preparations in the late luteal phase to postpone menses (de Ziegler *et al.*, 1998). In addition, minimal hyperstimulation may be even less effective than current strategies in women with a reduced ovarian response due to advanced reproductive age. The overall effectiveness of minimal stimulation protocols remains to be established in large series of patients.

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