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Mild ovarian stimulation for IVF

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BACKGROUND: Mild ovarian stimulation for *in vitro* fertilization (IVF) aims to achieve cost-effective, patient-friendly regimens which optimize the balance between outcomes and risks of treatment.

METHODS: Pubmed and Medline were searched up to end of January 2008 for papers on ovarian stimulation protocols for IVF. Additionally, references to related studies were selected wherever possible.

RESULTS: Studies show that mild interference with the decrease in follicle-stimulating hormone levels in the mid-follicular phase was sufficient to override the selection of a single dominant follicle. Gonadotrophin-releasing hormone antagonists compared with agonists reduce length and dosage of gonadotrophin treatment without a significant reduction in the probability of live birth (OR 0.86, 95% CI 0.72–1.02). Mild ovarian stimulation may be achieved with limited gonadotrophins or with alternatives such as anti-estrogens or aromatase inhibitors. Another option is luteinizing hormone or human chorionic gonadotrophin administration during the late follicular phase. Studies regarding these approaches are discussed individually; small sample size of single studies along with heterogeneity in patient inclusion criteria as well as outcomes analysed does not allow a meta-analysis to be performed. Additionally, the implications of mild ovarian stimulation for embryo quality, endometrial receptivity, cost and the psychological impact of IVF treatment are discussed.

CONCLUSIONS: Evidence in favour of mild ovarian stimulation for IVF is accumulating in recent literature. However, further, sufficiently powered prospective studies applying novel mild treatment regimens are required and structured reporting of the incidence and severity of complications, the number of treatment days, medication used, cost, patient discomfort and number of patient drop-outs in studies on IVF is encouraged.

Introduction

Ovarian stimulation has become a key component of assisted reproductive technologies (ART). For 25 years, ovarian stimulation has been applied with the aim of increasing the number of oocytes in order to compensate for inefficiencies of the *in vitro* fertilization (IVF) procedure enabling the selection of one or more embryos for transfer (Fauser *et al.*, 2005). At the present time, a long gonadotrophin-releasing hormone (GnRH) agonist pituitary suppression regimen combined with relatively high doses of exogenous follicle-stimulating hormone (FSH) remains the most frequently used stimulation protocol (FIVNAT, 1997; Macklon *et al.*, 2006). Gonadotrophin starting doses usually vary between 150 and 450 IU/day, although several randomized trials have failed to demonstrate improvements in outcome when higher doses are employed (van Hooff *et al.*, 1993; Hoomans *et al.*, 1999, 2002; Out *et al.*, 2000,

2001; Latin-American Puregon IVF Study Group, 2001; Wikland *et al.*, 2001; Yong *et al.*, 2003).

Currently used medication regimens for ovarian stimulation are complex, expensive, may require weeks of daily injections and intense ovarian response monitoring is usually needed. Such regimens are associated with the risk of complications such as ovarian hyperstimulation syndrome (OHSS) (Fauser *et al.*, 1999; Delvigne and Rozenberg, 2002; Aboulghar and Mansour, 2003). Other negative effects associated with ovarian stimulation include emotional stress, high drop-out rates and abdominal discomfort (Fauser *et al.*, 2005). Moreover, uncertainties remain regarding long-term health risks (such as ovarian cancer) and an increased incidence of low birthweight and birth defects in the offspring conceived following IVF treatment (Hansen *et al.*, 2002; Olivennes, 2002; Wang *et al.*, 2005; Kapiteijn *et al.*, 2006).

In 1996, Edwards *et al.* were the first to express concern with regard to contemporary ovarian stimulation approaches for IVF and called for the use of milder stimulation protocols (Edwards *et al.*, 1996). The aim of mild stimulation is to develop safer and more patient-friendly protocols in which the risks of treatment are minimized (Diedrich and Ferberbaum, 1998; Olivennes and Frydman, 1998; Fauser *et al.*, 1999; Olivennes *et al.*, 2002; Nargund and Frydman, 2007; Pennings and Ombelet, 2007; Ubaldi *et al.*, 2007) (Table I).

A potential concern regarding the application of milder stimulation protocols in routine clinical practice is that a decreased ovarian response following mild stimulation will reduce pregnancy rates. However, increased efficacy of IVF laboratory procedures and the current tendency—in some parts of the world—to limit the number of embryos transferred, has reduced the need for large quantities of oocytes. Furthermore, supportive evidence regarding a potentially negative effect of supraphysiological steroid levels on endometrial receptivity (Simon *et al.*, 1995; Devroey *et al.*, 2004), corpus luteum function (Fauser and Devroey, 2003; Beckers *et al.*, 2006), oocyte and embryo quality (Valbuena *et al.*, 2001; Baart *et al.*, 2007) indicate that limited ovarian stimulation and response might have a beneficial effect upon implantation potential.

Table I Considerations related to different approaches in ovarian stimulation

Current ovarian stimulation approaches
Aiming for maximum number of oocytes
Time consuming and complex stimulation regimens
High costs
Much patient discomfort
Short-term complications—ovarian hysterstimulation syndrome (OHSS)
Long-term health consequences uncertain
High drop-out rates
Supraphysiological steroid levels with possible implications
Emphasize additional pregnancy chances from cryopreserved embryos
Emphasize maximizing pregnancy rates per cycle
Mild stimulation approaches
Less complex
Less time consuming
Cheaper (making IVF more accessible for a broader patient population)
Reduced chances for complications
Reduced chances for discomfort
Reduced chances for drop-out
Effects on oocyte quality
Effects on endometrial receptivity
Emphasize maximizing chances for healthy children born per started treatment at reasonable cost, patient discomfort and chances for complications

Methods

This literature review will discuss the rationale behind milder ovarian stimulation approaches and the evidence regarding the efficacy of these protocols. In order to make a complete overview Pubmed and Medline were searched up to the end of January 2008 using the keywords IVF, ovarian stimulation protocol, mild and minimal stimulation and (modified) natural cycle. Additional searches were made using stimulation specific medications used, e.g. clomiphene citrate (CC), luteinizing hormone (LH)/FSH and aromatase inhibitors. References were selected which reported on related work whenever possible.

Relevant physiology of follicle development

Complete follicular development takes over 220 days and can be classified into three phases according to the developmental stage and the follicular gonadotrophin dependence. First, the initial recruitment of resting primordial follicles, second the development of preantral and early antral follicles and finally cyclic recruitment of a limited cohort of antral follicles followed by the selection of a single dominant follicle during the mid-follicular phase of the menstrual cycle (Gougeon, 1996; Fauser and van Heusden, 1997; McGee and Hsueh, 2000) (Fig. 1).

In the adult ovary, folliculogenesis starts when follicles leave the pool of resting follicles to enter the growth phase. The size of the follicle pool is determined during fetal life and reaches its maximum of 6–7 million by 20 weeks of gestation (Baker, 1963). From this point in time, germ cell content will decrease due to a continuous flow of follicles leaving the primordial follicle pool (initial recruitment). Around 1000 primordial follicles start growing every month. The exact mechanism underlying the initiation of growth is not well understood and appears to be under the control of intra-ovarian autocrine and paracrine factors (Gougeon, 1996; Fortune *et al.*, 2000). The great majority of primordial follicles that enter this development phase undergo atresia before reaching the antral follicle stage, principally through apoptosis (McGee and Hsueh, 2000).

After initial recruitment, follicles entering the growth phase enlarge, both by proliferation and differentiation of granulosa cells and an increase in the size of the oocyte. The time span of the development from primary recruitment to the early antral follicle stage in humans is unknown but is proposed to be several months. During early preantral follicle development, FSH receptors become detectable on granulosa cells. Although at this stage the follicle seem unaffected by the absence of gonadotrophins [as shown in women diagnosed with

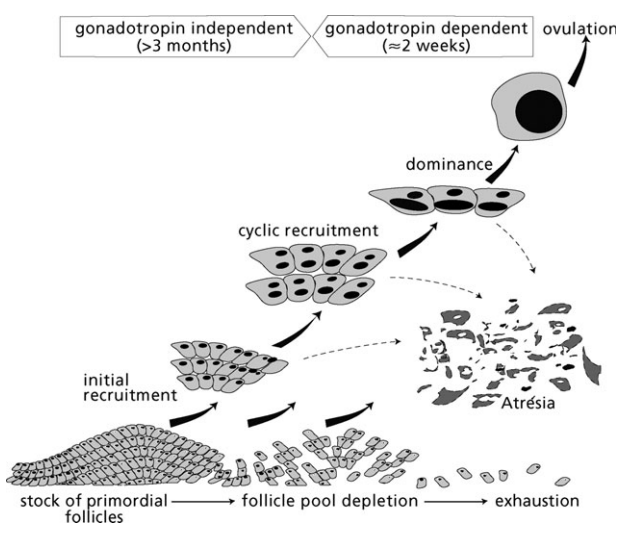

Figure 1 Schematic representation of life history of ovarian follicles: endowment and maintenance, initial recruitment, maturation, atresia or cyclic recruitment, ovulation, and exhaustion. Adapted from McGee and Hsueh (2000).

Kallmann syndrome, or after hypophysectomy (Schoot *et al*., 1992)], growth may be stimulated by the presence of FSH (McGee and Hsueh, 2000).

In contrast to the early stages of follicle development, the presence of FSH is an absolute requirement for the development of larger antral follicles. From this point onwards, FSH acts as a survival factor for antral follicles, which are being rescued from atresia by the intercycle rise in serum FSH level (Fauser and van Heusden, 1997). Although each growing follicle may initially have an equal potential to reach full maturation, only those follicles continue to grow that are at a more advanced developmental stage (2–5 mm in diameter) at the time FSH levels surpass the threshold during the luteo-follicular transition. The number of follicles available for cyclic recruitment is dependent on the age of the women and is estimated to be around 11 per ovary (Hodgen, 1982; Pache *et al*., 1990) (Fig. 2).

After the initial rise, FSH concentrations plateau during the early follicular phase and finally decrease during the mid to late follicular phase as a consequence of inhibin B and ovarian steroid negative feedback (Zeleznik *et al*., 1985; Groome *et al*., 1996; Schipper *et al*., 1998). The decrease in FSH limits the time that the FSH concentration is above the threshold, which appears to be essential for single dominant follicle selection (van Santbrink *et al*., 1995) (Fig. 2). Despite the decline in FSH, the most mature follicle continues its growth due to its increased sensitivity for FSH and acquired responsiveness to LH

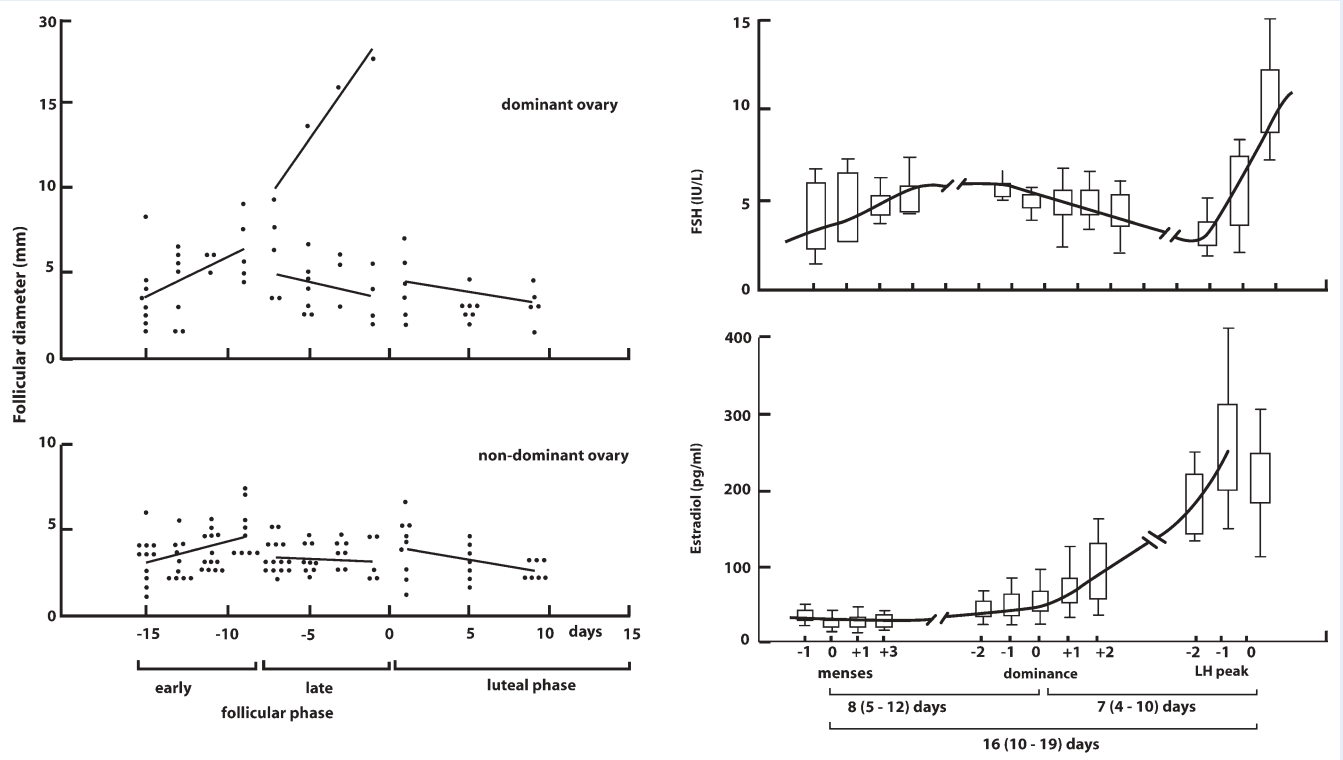

Figure 2 (Left) Representation of number and size of antral follicles as assessed by transvaginal ultrasound during the menstrual cycle of a normal cycling woman (Day 0 = LH surge) (Pache *et al*., 1990).(Right) Box and whisker plots representing serum FSH (upper panel) and estradiol (lower panel) concentration in 16 regularly menstruating female volunteers, synchronized around the initiation of menses, around the first day of visualization of a dominant follicle, and preceding the mid-cycle LH peak (van Santbrink *et al*., 1995).

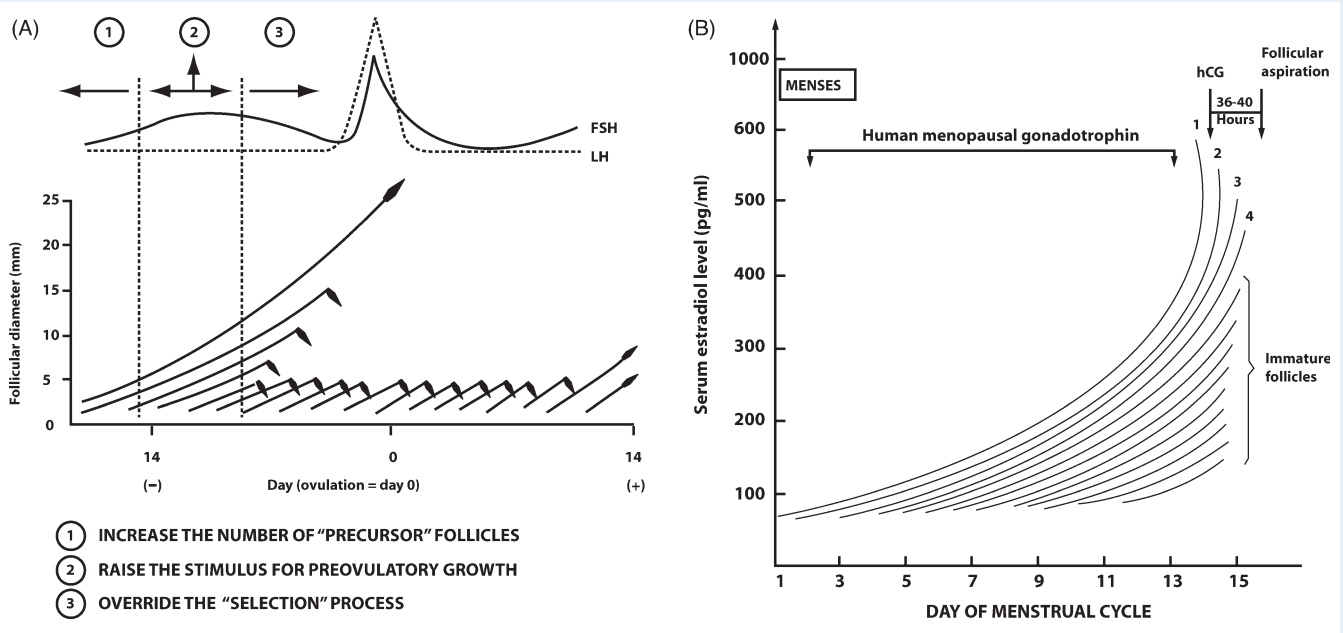

Figure 3 (A) Schematic representation of serum FSH levels and number and size of ovarian follicles during ovarian stimulation for IVF (Hillier *et al.*, 1985). (B) Schematic representation of the heterogeneous cohort of recruited and selected follicles in HMG-stimulated cycles for IVF (Oehninger and Hodgen, 1990).

(Hillier, 1994; McGee and Hsueh, 2000). All other recruited follicles lack sufficient FSH stimulation and enter atresia. The 'FSH gate' (Baird, 1987) or 'FSH window' (Fauser *et al.*, 1993) concept introduces the element of time rather than the magnitude of the FSH rise to the FSH threshold theory. The window concept emphasizes the importance of a transient increase of FSH above the threshold level in order to gain single dominant follicle selection. Ovarian stimulation makes use of the concept that disruption of the decline of FSH levels leads to the development of multiple dominant follicles (Fig. 3).

After exogenous gonadotrophins became available, growth of multiple dominant follicles was accomplished by the administration of high doses of gonadotrophins during the entire follicular phase (Hillier *et al.*, 1985; Oehninger and Hodgen, 1990) (Fig. 3). However, a later study in primates showed that mild interference with the decrease in FSH levels during the mid-follicular phase is sufficient to override the selection of a single dominant follicle (Zeleznik *et al.*, 1985). Subsequently, this concept was confirmed in humans; a moderate, but continued, elevation of FSH levels during the mid to late follicular phase (effectively preventing decremental FSH concentrations) was sufficient to interfere with single dominant follicle selection and induces ongoing growth of multiple follicles in normo-ovulatory volunteers (Fig. 4) (Schipper *et al.*, 1998) and (Fig. 5) (Hohmann *et al.*, 2001).

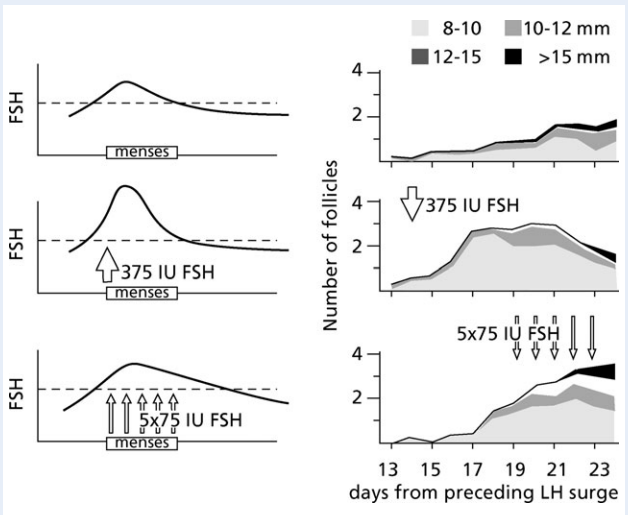

Figure 4 FSH window concept with mild intervention approaches, stressing the significance of the limited duration of FSH elevation above the threshold level rather than the height of the elevation of FSH for multiple dominant follicle selection.The left figures show the intervention, and the right figures show the resulting number of follicles during the follicular phase in normo-ovulatory female volunteers. (Upper) Natural cycle with single dominant follicle selection. (Middle) Intervention cycle with administration of a single s.c. injection of 375 IU FSH on Day LH+14. (Under) Intervention cycle with five s.c. injections of 75 IU FSH daily from Day LH+19 until Day LH+23. Adapted from Schipper *et al.* (1998).

The development of milder stimulation protocols

Introduction of GnRH antagonists

The introduction of GnRH antagonists into clinical practice has allowed for the introduction of milder stimulation approaches for

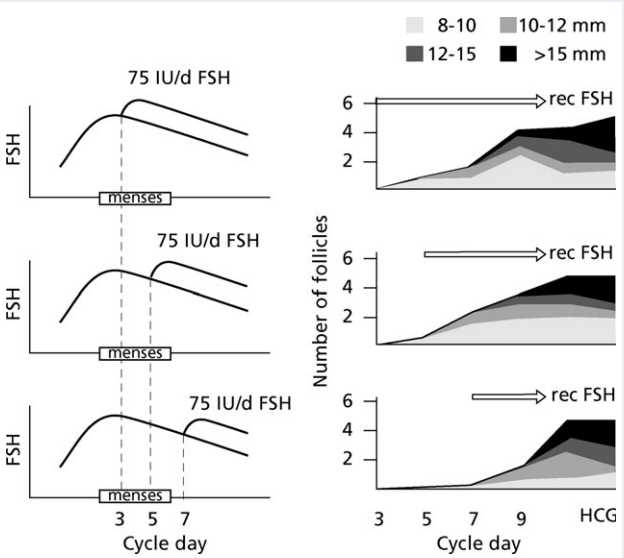

Figure 5 Multi-follicular development in normo-ovulatory female volunteers receiving fixed low daily doses (75 IU) of exogenous FSH starting on either CD 3, 5 or 7. The left figures show the intervention, and the right figures show the resulting number of follicles during the follicular phase. Adapted from Hohmann *et al.* (2001).

IVF treatment (Tarlatzis *et al.*, 2006). GnRH antagonists prevent the premature LH rise by competitive blockade of the GnRH receptor. Unlike GnRH agonists, GnRH antagonists do not induce an initial flare of endogenous gonadotrophin release, but cause an immediate and rapid, reversible suppression of gonadotrophin secretion. The use of GnRH antagonist exclusively during the mid to late stimulation phase (the period at risk for a premature rise in LH) therefore allows for the initiation of the IVF treatment cycle in a normal menstrual cycle with an undisturbed recruitment of a cohort of follicles during the early follicular phase. This approach enables the endogenous inter-cycle FSH rise to be utilized rather than suppressed, resulting in a reduction of medication needed. The use of ovarian stimulation in the normal menstrual cycle also enables more IVF cycles to be carried out in a given period than is possible with a long GnRH agonist stimulation protocol.

Three general approaches for GnRH antagonist co-treatment have emerged. A single large dose can be injected subcutaneously on approximately the eighth day of stimulation with gonadotrophins. Alternatively, daily injections of small doses could be initiated on a fixed day of stimulation (usually Day 6) or depending on the size of the dominant follicle or the estradiol level (flexible protocol) and continued until the day that human chorionic gonadotrophin (hCG) for final oocyte maturation is given (for review Huirne and Lambalk, 2001).

As was shown in a meta-analysis of 27 IVF studies, the use of GnRH antagonist co-treatment compared with agonist long protocol leads to a considerable reduction in the number of days GnRH analogue treatment is needed [weighted mean difference (WMD) −20.90, 95% CI −22.20 to −19.60], the number of days of gonadotrophin administration (WMD −1.54, 95% CI −2.42 to −0.66), the amount of gonadotrophin ampoules used (WMD −4.27, 95% CI −10.19 to 1.65) and the incidence of severe OHSS (RR 0.61, 95% CI 0.42–0.89) (Al-Inany *et al.*, 2006).

Moreover, the use of GnRH antagonists is not complicated by cyst formation due to the GnRH agonist flare-up effect. Although initial studies suggested a detrimental effect on pregnancy rates following GnRH antagonist compared with agonists (Ludwig *et al.*, 2001; Al-Inany *et al.*, 2006; Tarlatzis *et al.*, 2006), a recent meta-analysis including 22 randomized controlled trials (RCT) involving 3176 subjects showed no significant difference in the probability of live birth (OR 0.86, 95% CI 0.72–1.02) (Kolibianakis *et al.*, 2006).

To date, GnRH agonists remain in use in the majority of clinics. This is probably due to the established position of GnRH agonist in standard regimens (Kolibianakis *et al.*, 2005), initial reports on a possible reduction in pregnancy rates (Al-Inany *et al.*, 2006) and the reduced flexibility in the programming of IVF cycles with GnRH antagonist co-treatment (Fauser and Devroey, 2005).

Natural cycle and modified natural cycle with FSH add-back

The first successful IVF treatment was performed in an unstimulated menstrual cycle (Steptoe and Edwards, 1978). Soon thereafter, IVF in natural cycles was largely replaced by ovarian stimulation to improve the success rate per cycle (Trounson *et al.*, 1981; Cohen *et al.*, 2005; Macklon *et al.*, 2006). Natural cycle IVF in its basic form consists of simply monitoring the spontaneous cycle, and retrieving a single oocyte prior to the spontaneous LH peak. Consequently, the chance for multiple pregnancies and OHSS are minimal. Natural cycle IVF is physically less demanding, requiring no or far less hormonal medication. The per-cycle costs of natural cycle IVF have been calculated to be 20–23% of those of stimulated IVF (Aboulghar *et al.*, 1995; Nargund *et al.*, 2001). Ongoing pregnancy rates per started natural cycle IVF have been reported to be 7.2%, which seems unacceptably low for most patients despite being less stressful. However, this may vary according to the population studied (for review Pelinck *et al.*, 2002).

Natural cycle IVF results are hampered by high cancellation rates due to premature LH rises, premature ovulation or reduced chances for successful oocyte retrieval (Pelinck *et al.*, 2002). The planning of oocyte retrieval based on a LH rise requires frequent monitoring and round-the-clock oocyte retrieval and laboratory facilities. The use of hCG for the triggering of final oocyte maturation, and indomethacin to postpone follicle rupture (Nargund *et al.*, 2001) allows for a certain degree of planning. Flushing of the follicle during oocyte retrieval (Bagtharia and Haloob, 2005) may increase the efficacy of the procedure.

Only four RCTs involving a total of 339 women comparing natural cycle IVF with stimulated IVF cycles have been published so far (Table II). The outcome of natural cycle IVF was compared with IVF in CC-stimulated cycles (MacDougall *et al.*, 1994; Ingerslev *et al.*, 2001), human menopausal gonadotrophin (HMG)/GnRH agonist long protocol cycles (Levy *et al.*, 1991) or with IVF cycles combining purified FSH ovarian stimulation with a GnRH agonist microdose flare protocol (Morgia *et al.*, 2004). Despite relatively small numbers of patients, and variable numbers of treatment cycles per patient, natural cycle IVF was consistently observed to result in lower pregnancy rates (Table II).

Table II Characteristics of randomized controlled trials involving natural cycle IVF

Study	Inclusion criteria	Study protocol	Control stimulation protocol	Main outcome
Levy *et al.* (1991) (abstract)	Patients with regular ovulatory menstrual cycles and no male factor	Natural cycle IVF with hCG when the leading follicle was ≥16 mm and E2 ≥160 pg/ml (22 cycles)	Long GnRH agonist protocol with HMG (26 cycles)	Cancellation rate 27 versus 4%. Ongoing pregnancy rate 0 versus 23% ($P < 0.01$)
MacDougall *et al.* (1994)	Patients ≤38 years with >1 year of infertility, spontaneous ovulatory regular cycles and normal semen analysis	Natural cycle IVF with hCG when the leading follicle was 17 mm ($n = 14$)	CC 100 mg, from Days 2–6, hCG when the leading follicle was 17 mm ($n = 16$)	Cancellation rate 71 versus 0%. Ongoing pregnancy rate 0 versus 13% (NS)
Ingerslev *et al.* (2001)	Couples with no previous IVF attempts under 35 years with ICSI indication, tubal factor or idiopatic infertility	Natural cycle IVF with hCG when the leading follicle was≥17 mm (64 patients, 114 cycles)	CC 100 mg, from Days 3–7 and hCG when the leading follicle was ≥20 mm (68 patients, 111 cycles)	Cycles resulting in embryo transfer 25.4 versus 53.2%. Ongoing pregnancy rate (per cycle) 3.5 versus 18.0% ($P < 0.001$)
Morgia *et al.* (2004)	Poor-responding patients (<4 follicles in a previous IVF attempt) with a regular menstrual cycle. ICSI was performed in all cycles	Natural cycle IVF with hCG when the leading follicle was ≥16 mm (59 patients, 114 cycles)	GnRH analog flare protocol with 0.05 mg buserelin twice daily from Day 1 and 600 IU purified FSH/day from Day 3 (70 patients, 101 cycles)	Cycles resulting in embryo transfer 41.2 versus 68.3%. Ongoing pregnancy rate (per cycle) 6.1 versus 6.9% (NS)

The number of included cycles is equal to the number of included patients unless stated otherwise. Outcomes were significantly different unless stated otherwise. Pregnancy rates are given per started cycle unless stated otherwise.

To improve effectiveness, natural cycle IVF could be offered as a series of treatment cycles, for it is safer, less stressful compared with conventional stimulation. It has been postulated that after four cycles of natural cycle IVF, the cumulative probability of pregnancy is ~46% with an associated live birth rate of 32% in a selected groups of patients (Nargund *et al.*, 2001). In this study, 52 patients with a regular menstrual cycle underwent 181 natural cycle IVF attempts which resulted in 16 live births. Even though the outcome of four cycles of natural cycle IVF was found to be comparable to a single cycle of IVF with ovarian stimulation and being cost-effective, the added investment of time and increased number of oocyte retrieval procedures also should be taken into account.

To improve outcomes while preserving the advantages of natural cycle IVF, modifications have been made. In the 'modified' natural cycle, the occurrence of a premature LH rise is prevented by the use of a GnRH antagonist during the late follicular phase. The ongoing growth of the dominant follicle is supported by the addition of exogenous gonadotrophins (referred to as 'add back'). In most studies, GnRH antagonist and gonadotrophins (75–300 IU/day) are initiated at a follicle diameter of 12–17 mm.

Up to the present, no RCTs studying the efficacy of modified natural cycle IVF have been published. Most studies regarding modified natural cycle IVF include patients with a previous poor response to conventional ovarian stimulation. In this population, success rates between 0 and 14%per started cycle have been reported in non-randomized studies (Elizur *et al.*, 2005; Castelo-Branco *et al.*, 2004; Kolibianakis *et al.*, 2004; Weghofer *et al.*, 2004; Hur *et al.*, 2005). One large cohort study analysed the cumulative pregnancy rate after three modified natural IVF cycles in good prognosis patients (Pelinck *et al.*, 2006). A total of 844 treatment cycles in 350 patients of <36 years of age with no previous IVF treatment were included. The ongoing pregnancy rate per cycle was 8.3 and 20.8% after up to three cycles. The number of cancelled cycles related to a rise in LH or ovulation in this study was 13% per started cycle, compared with an average of 20% reported following natural cycle IVF.

Relatively high pregnancy rates have been reported in young couples with severe male infertility as the only fertility compromising factor. In this category of patients, the success rate per started cycle was 13.3% (Zhioua *et al.*, 2004) and cumulative pregnancy rates of 43.8% after six successive cycles (Vogel *et al.*, 2003) have been reported.

These studies show that (modified) natural cycle is a safe and patient friendly treatment option. Despite the advantages of this approach, low efficacy has restricted its widespread use. Modified natural cycle IVF in consecutive cycles in a selected population may result in improved effectiveness.

Clomiphene citrate

The anti-estrogen CC was the first preparation used for ovarian stimulation in IVF (Trounson *et al.*, 1981; Quigley *et al.*, 1984; Cohen *et al.*, 2005). CC has now been largely replaced by more effective HMG/FSH protocols in combination with GnRH analogue co-treatment (Fraser and Baird, 1987; Macklon *et al.*, 2006). Important advantages of CC compared with gonadotrophins remain including its oral administration, low price and widespread availability. CC acts to increase pituitary FSH secretion by reducing negative estrogen feedback.

An ovarian stimulation protocol combining CC with gonadotrophins could lead to a reduction in the amount of gonadotrophins required due to the combined synergistic effects. Additionally, because gonadotrophins may counterbalance the undesired anti-estrogenic effects of the CC on the endometrium (Markiewicz *et al.*, 1988; Nelson *et al.*, 1990) which has been held responsible for the relatively low embryo implantation rates observed this combination might lead to improved pregnancy rates compared with CC alone.

Two randomized trials have compared the outcome of CC/gonadotrophin treatment cycles with a standard long GnRH agonist

ovarian stimulation protocol. In one study, significantly higher cycle cancellation rates and lower pregnancy rates per cycle were observed following a CC protocol in combination with 150 IU HMG ($P = 0.002$) (Dhont *et al*., 1995). On the other hand, stimulation with goserelin/HMG was associated with a higher number of ampoules of HMG (44.9 versus 9.9; $P < 0.0001$) and a longer duration of stimulation (11.2 versus 8.7 days; $P < 0.0001$). A more recent study involving similar patient numbers, concluded that a stimulation regimen combining CC with 225 IU FSH and 75 IU of recombinant LH (rLH) on alternate days resulted in comparable cancellation and ongoing pregnancy rates per cycle to those following a standard long GnRH agonist protocol (Weigert *et al*., 2002). The average number of ampoules of FSH used was significantly reduced in the CC group (13.9 versus 16.6 ampoules; $P < .0001$). The characteristics of the RCTs on the various CC protocols for IVF treatment have been summarized in Table III.

The recent availability of GnRH antagonists has allowed for the prevention of premature LH rises in combination with CC. One randomized controlled study showed that a CC/gonadotrophin regimen with GnRH antagonist co-treatment resulted in similar pregnancy outcomes compared with a standard long GnRH agonist stimulation protocol while significantly reducing the number of ampoules HMG used, the number of treatment days and the number of oocytes retrieved (Lin *et al*., 2006). This study confirmed the findings of two earlier retrospective analyses which concluded that equally high pregnancy rates could be obtained with a CC/gonadotrophin protocol with GnRH antagonist co-treatment compared with standard ovarian stimulation, with a significant reduction in the total dose of gonadotrophins needed (Williams *et al*., 2002; Fiedler and Ludwig, 2003). In contrast, a non-randomized comparative study observed significantly lower pregnancy rates following ovarian stimulation with a CC/HMG protocol with GnRH antagonist co-treatment compared with a long GnRH agonist protocol (Mansour *et al*., 2003). Whether the addition of GnRH antagonist to the CC/gonadotrophin protocol improves outcomes remains unclear. A randomized controlled study observed similar ongoing pregnancy rates with and without the use of GnRH antagonist (Fiedler *et al*., 2001). The need for pituitary suppression in combination with CC is probably dependent on the dosage of medication used as well as individual differences in ovarian response.

In most studies, gonadotrophins are combined with CC in a dose of 100 mg/day for 5 days during the early follicular phase. However, a high rate of heterogeneity exists in studies concerning the optimal use of the other components of this stimulation approach. It has been debated whether HMG or FSH with or without rLH supplementation should be used (Engel *et al*., 2002; Weigert *et al*., 2002). Additionally, there is no evidence regarding

Table III Characteristics of randomized controlled trials involving ovarian stimulation with clomiphene citrate for IVF

Study	Inclusion criteria	Study protocol	Control stimulation protocol	Main outcome
MacDougall *et al*. (1994)	Patients ≤38 years with >1 year of infertility, spontaneous ovulatory regular cycles and normal semen analysis	CC 100 mg, from Days 2–6, hCG when the leading follicle was 17 mm ($n = 16$)	Natural cycle IVF with hCG when the leading follicle was 17 mm ($n = 14$)	Cancellation rate 0 versus 71%. Ongoing pregnancy rate 13 versus 0% (NS)
Dhont *et al*. (1995)	Patients with no previous IVF attempts. Treatment included IVF-ET, ZIFT and GIFT	OAC pretreatment, CC 100 mg for 5 Days and (150) subsequent HMG ($n = 151$)	OAC pretreatment, long acting GnRH agonist and (300 IU) HMG ($n = 152$)	Cancellation rate 20.5 versus 2.6%. Ongoing pregnancy rate 24.5 versus 36.8% ($P = 0.02$)
Ingerslev *et al*. (2001)	Couples with no previous IVF attempts under 35 years with ICSI indication, tubal factor or idiopathic infertility	CC 100 mg, from Days 3–7 and hCG when the leading follicle was ≥20 mm (68 patients, 111 cycles)	Natural cycle IVF with hCG when the leading follicle was ≥17 mm (64 patients, 114 cycles)	Cycles resulting in embryo transfer 53.2 versus 25.4%. Ongoing pregnancy rate (per cycle) 18.0versus 3.5% ($P < 0.001$)
Fiedler *et al*. (2001) (abstract)	Random selected normal cycling women	100 mg CC CD 5–9, from Day 9 additional 150 IU HMG or FSH. GnRH antagonist from Day 10 ($n = 295$)	100 mg CC CD 5–9, from Day 9 additional 150 IU HMG or FSH ($n = 291$)	Ongoing pregnancy rate 23 versus 21% (NS)
Weigert *et al*. (2002)	Women with no previous IVF cycles, between 20 and 39 years, with normal ovulatory cycles with tubal, male factor or unexplained infertility	OAC pretreatment. CC 100 mg for 5 days in combination with 225 IU of rFSH and 75 IU of rLH on alternate days ($n = 154$)	Long GnRH suppression and 150 IU rFSH ($n = 140$)	Ongoing pregnancy rate 35 versus 29% (NS)
Engel *et al*. (2003)	Healthy female partners of infertile couples, between 18 and 39 years, with regular cycle length. No more than three previous IVF cycles or basal FSH >10 IU/l	Single dose GnRH antagonist protocol. CC 100 mg CD 2–6 of 3–7, CD 6 start 150 IU rFSH ($n = 5$)	Single dose GnRH antagonist protocol. CC 100 mg CD 2–6 of 3–7, CD 6 start 150 IU HMG ($n = 5$)	Live birth rate 40 versus 20% (NS)
Lin *et al*. (2006)	Couples with male-factor infertility who were about to undergo their first ICSI cycle	CC/HMG. Cetrorelix protocol ($n = 60$)	buserelin long protocol ($n = 60$)	Pregnancy rate 41.7 versus 40% (NS)

The number of included cycles is equal to the number of included patients unless stated otherwise. Outcomes were significantly different unless stated otherwise.

the optimal gonadotrophin regimen; most studies vary in the starting dose, day of initiation, daily injections or on alternate days or as a single bolus of long acting FSH (Corfman *et al*., 1993; Obruca *et al*., 1993; Engel *et al*., 2002; Tavaniotou *et al*., 2003; D'Amato *et al*., 2004; Kawachiya *et al*., 2006).

In conclusion, more studies are required to optimize the CC/gonadotrophin stimulation protocol. The heterogeneity in the studies thus far published does not allow to draw conclusions to be drawn regarding the possible benefits of CC in ovarian stimulation for IVF. However, given its low cost, CC may have a place in cost-effective mild ovarian stimulation treatments.

Aromatase inhibitors

Aromatase inhibitors selectively inhibit the conversion of androgens to estrogens in granulosa cells of developing ovarian follicles, resulting in a subsequent increase in intra-ovarian androgens and absence of a rise in estrogens (Garcia-Velasco *et al*., 2005). Intra-ovarian androgens may have a profound effect on early follicle growth and increase the number of preantral and small antral follicles as androgens stimulate theca and granulosa cell proliferation and inhibit apoptosis (Vendola *et al*., 1998). Due to a reduced estrogen feedback and resulting increased endogenous gonadotrophin secretion, the need for exogenous gonadotrophins is likely to be reduced when aromatase inhibitors are administered in the early follicular phase. Aromatase inhibitors may therefore serve a similar purpose as CC. Like CC, aromatase inhibitors are orally taken and are cheap. However, compared with CC they offer the potential advantage of not causing depletion of estrogen receptors (Mitwally and Casper 2001, 2003) and are more rapidly cleared from the body because of their shorter half-life (~45 h instead of a few weeks). In theory, significantly reduced intrafolliular estrogen concentrations may impact on oocyte quality which may affect IVF outcomes.

Aromatase inhibitors have been in clinical use for more than 20 years, primarily in the treatment of advanced breast cancer in postmenopausal patients (Winer *et al*., 2002). The use of these compounds have only recently been introduced in infertility treatment, especially for ovulation induction (Casper and Mitwally, 2006) and as a mild and safe ovarian stimulation method for IVF treatment in patients with breast cancer (Oktay *et al*., 2003, 2005). Recent data have raised concerns regarding possible teratogeneity of aromatase inhibitors (Biljan *et al*., 2005) and ovarian stimulation is currently an off-label use again marketer's advice. Even though these findings were not confirmed in a larger group of patients (Tulandi *et al*., 2006), animal studies have shown toxic effects on prenatal development in rats after exposure to letrozole *in utero* (Tiboni *et al*., 2008).

There are limited clinical data available concerning the use of aromatase inhibitors in IVF treatment. One preliminary uncontrolled study observed an ongoing pregnancy rate of 27% following the use of aromatase inhibitors as a cheap treatment alternative in 22 good prognosis patients with limited financial means (Grabia *et al*., 2006). In this study, HMG was initiated on cycle Day (CD) 7 after 5e days of letrozole (2.5 mg CD 3–7) with GnRH antagonist co-treatment.

To date, only three RCTs involving a total of 80 women have studied the use of aromatase inhibitors in IVF. However, aromatase inhibitors were administered in combination with a standard rather than mild ovarian stimulation protocol in all three studies. In two trials, aromatase inhibitors were added to a standard treatment schedule using high doses of gonadotrophins in patients with a poor response in a previous treatment cycle (Goswami *et al*., 2004; Kahraman *et al*., 2005). Both studies showed no benefit although the study groups were too small to draw meaningful conclusions. The third study randomized 20 good prognosis patients for the use of 150 IU rFSH from CD 2 with or without the addition of 2.5 mg letrozole and GnRH antagonist co-treatment from CD 6 (Verpoest *et al*., 2006). The use of aromatase inhibitors resulted in higher numbers of oocytes and a tendency towards higher clinical pregnancy rates per started cycle in the letrozole group. In conclusion, more sufficiently powered randomized studies are needed to assess the true benefit of aromatase inhibitors in IVF treatment.

Exogenous gonadotrophins with GnRH antagonist co-medication

Mild ovarian stimulation in which low-dose gonadotrophin (FSH/HMG) administration is delayed until the mid-follicular phase is based on the FSH window concept (Fauser *et al*., 1993, 1997). Exogenous FSH administration is limited to the mid to late follicular phase with the aim of preventing a decrease of FSH levels and thus inducing multi-follicular development (Fig. 4) (Schipper *et al*., 1998). The availability of GnRH antagonists for acute suppression of a premature LH rise enabled this concept to be introduced into IVF (Macklon and Fauser, 2000). A pilot study showed that multiple dominant follicles could even be induced when the initiation of FSH was postponed until CD 7 (de Jong *et al*., 2000). However, there was a tendency toward a lower percentage of women presenting with multiple dominant follicle development compared with patients started on CD 3 or 5 (Fig. 5) (Hohmann *et al*., 2001). A fixed daily dose of 150 IU rFSH compared with 100 IU/day was found to be more effective in consistently inducing multiple follicular growth when ovarian stimulation was initiated on CD 5 (de Jong *et al*., 2000).

In a prospective randomized study involving 142 patients, the efficacy of a stimulation protocol initiating ovarian stimulation (150 IU/day) on CD 5 (with GnRH antagonist co-treatment from a follicle size of 14 mm) was compared with a conventional long GnRH agonist protocol and a standard GnRH antagonist protocol with an early follicular phase start of FSH (Hohmann *et al*., 2003). This study concluded that the tested mild protocol resulted in pregnancy rates per started cycle comparable to those observed following conventional ovarian stimulation with GnRH agonist co-treatment despite a reduced duration of stimulation and a marked reduction in the amount of exogenous FSH needed ($P < 0.001$ and 0.02, respectively).

To investigate the use of this mild stimulation protocol in clinical practice, a large randomized efficacy study was performed to analyse whether a mild strategy in IVF [combining mild ovarian stimulation with single embryo transfer (SET)] would lead to a similar overall outcome while reducing patients' discomfort, multiple pregnancies and costs compared with a standard treatment involving conventional stimulation and the transfer of two embryos (Heijnen *et al*., 2007). The study included a total of 404 patients (almost 800 consecutive IVF cycles) and observed that due to the shorter duration of treatment per cycle, less medication was needed and there was a reduction in twin pregnancies, the mild approach resulted in an equal cumulative chance of term live birth after a year of treatment while significantly

reducing the total costs and multiple birth (Fig. 6). Table IV shows the characteristics of four RCTs regarding a mild 'late start, low-dose' ovarian stimulation performed by our group.

Analysis of factors that influence the decision of couples to discontinue treatment showed that the use of a mild treatment strategy resulted in a significant reduction in drop-out rates (Verberg *et al*., 2008a). This finding shows that patients are willing to undergo more mild IVF treatment cycles as long as a mild treatment strategy is applied.

Late follicular phase hCG/LH

A stimulation protocol with late follicular phase replacement of FSH administration by LH has recently been proposed as an alternative mild stimulation approach. The replacement of FSH by LH is based on the acquired LH responsiveness of granulosa cells in dominant follicles (Hillier, 1994). In sheep, LH administration maintained elevated ovulatory rates despite FSH withdrawal (Campbell *et al*., 1999), while in humans the administration of rLH (300–750 IU/day) was found to be sufficient to maintain follicular growth in the late follicular phase after initial ovarian stimulation with exogenous gonadotrophins (Sullivan *et al*., 1999). Besides the expected reduction of gonadotrophin usage, this ovarian stimulation approach might also reduce the number of small, less mature follicles, conceivably reducing chances for OHSS (Filicori *et al*., 1999).

Three randomized trials comparing the late follicular phase hCG/LH protocol with the outcome of conventional stimulation protocols in patients with a favourable prognostic profile could be identified.

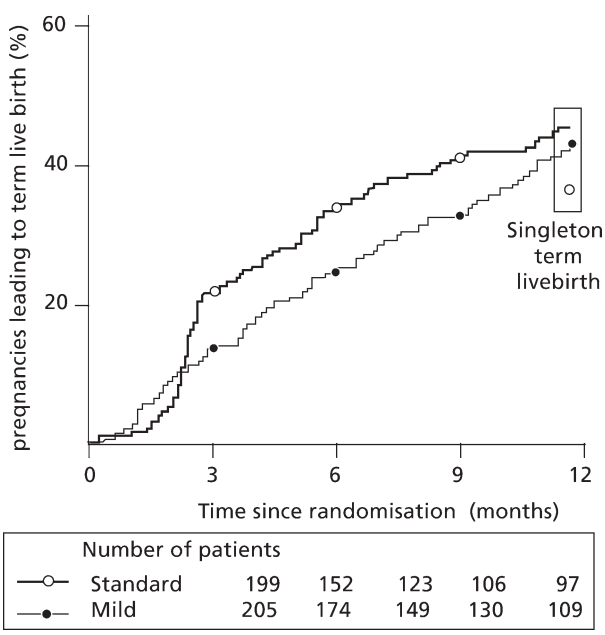

Figure 6 Cumulative term live-birth rate within 12 months after starting IVF treatment.Mild: mild ovarian stimulation with GnRH antagonist and single embryo transfer. Standard: standard ovarian stimulation with GnRH agonist long protocol along with the transfer of two embryos. The shaded area represents the singleton live birth rates after 12 months for which the study was designed and powered to compare (Heijnen *et al*., 2007).

In a large RCTs in 323 IVF patients the outcomes of a stimulation protocol with regular ovarian stimulation until CD 6, followed by a combination of 75 IU FSH and 200 IU hCG with GnRH antagonist co-treatment until oocyte retrieval were compared with a standard GnRH antagonist and a long GnRH agonist stimulation protocol (Serafini *et al*., 2006). The hCG protocol resulted in a significant reduction in rFSH needed. No difference in the number of (mature) oocytes obtained, ongoing pregnancy rates and incidence of OHSS. A similar design was applied in a study involving 109 patients, with the only exception that hCG was initiated when the largest follicle was 14 mm along with a fixed FSH dosage (Koichi *et al*., 2006). This study also observed similar pregnancy rates between the three groups, no difference in the number of oocytes or incidence of severe OHSS. However, a significant decrease in the total dose of gonadotrophins needed and small follicles at the time of final oocyte maturation was observed in the hCG group. Finally, the efficacy of a stimulation protocol with complete replacement of FSH with hCG from a follicular size of 12 mm in combination with a long GnRH agonist down-regulation protocol was studied (Filicori *et al*., 2005). This approach resulted in a significant reduction in FSH needed and less small follicles at final oocyte maturation in the hCG protocol without compromising the pregnancy rate, compared with a standard long GnRH agonist protocol. None of the studies reported untimely increments of follicular phase progesterone secretion or premature LH surges in the hCG/LH protocol.

These findings confirm that, in an selected group of patients, an ovarian stimulation protocol with late follicular phase hCG/LH stimulation leads to a reduced need of exogenous FSH and good pregnancy rates. However, despite the reported reduction in the number of small follicles, high estradiol levels were found and a reduced incidence of OHSS could not be established as yet. Additional studies are needed to determine the critical threshold for FSH replacement by LH stimulation, the most appropriate dosage of LH or hCG and establish the clinical benefit.

Implications of mild ovarian stimulation

Embryo quality

Some observations suggest that ovarian stimulation affects embryo quality as assessed by morphology as well as the chromosomal constitution of the embryos (Munne *et al*., 1997; Katz-Jaffe *et al*., 2005; Baart *et al*., 2007). This phenomenon could be the result of interference with the natural selection of good-quality oocytes or the exposure of growing follicles to the potentially negative effects of ovarian stimulation. Supportive evidence comes from several human and animal studies reporting detrimental effects of ovarian stimulation on oocyte and embryo quality.

An increased incidence of morphology and chromosomal abnormalities have been observed in oocytes after exposure to high doses of gonadotrophins during *in vitro* maturation of mouse oocytes (Eppig *et al*., 1998; van Blerkom and Davis, 2001; Roberts *et al*., 2005). Ovarian stimulation and concurrent high estradiol levels were shown to have a negative impact on the developmental and implantation potential of human embryos (Valbuena *et al*., 1999; Ertzeid and

Table IV Characteristics of randomized controlled trials involving mild 'late start' ovarian stimulation for IVF treatment.

Study	Inclusion criteria	Study protocol	Control stimulation protocol	Main outcome
De Jong *et al.* (2000)	Normo-ovulatory patients with a regular indication for IVF	From CD 5 ovarian stimulation with 100 IU/day FSH. GnRH antagonist from CD 8 or from leading foll 13 mm. No luteal support was provided ($n = 8$)	From CD 5 ovarian stimulation with 150 IU/day FSH. GnRH antagonist from CD 8 or from leading foll 13 mm. No luteal support was provided ($n = 7$)	Multiple follicle development 63 versus 100%. Ongoing pregnancy rate 25 versus 14% (NS)
Hohmann *et al.* (2003)	Normo-ovulatory patients with a regular indication for IVF (or IVF/ICSI)	Fixed FSH doses 150 IU/day from CD 5, GnRH antagonist from leading foll 14 mm ($n = 45$)	1. Fixed FSH doses 150 IU/day from CD 2, GnRH antagonist from leading foll 14 mm ($n = 48$). 2. Long GnRH agonist protocol, fixed FSH doses after 2 weeks 150 IU/day ($n = 49$)	Ongoing pregnancy rate 16 versus 17% (1.) versus 18% (2.) (NS)
Heijnen *et al.* (2007)	Regular cycling patients, below 38 years, BMI 19–29	Fixed FSH doses 150 IU/day from CD 5, GnRH antagonist from leading foll 14 mm. Combined with single embryo transfer (205 patients, 444 cycles)	Long GnRH agonist protocol, fixed FSH doses after 2 weeks 150 IU/day (199 patients, 325 cycles)	Ongoing pregnancy rate per year of treatment 47 versus 51% (NS)
Baart *et al.* (2007)	Regular cycling patients, below 38 years, BMI 19–29. Sperm count >5 million/ml. First cycles	Fixed FSH doses 150 IU/day from CD 5, GnRH antagonist from leading foll 14 mm ($n = 55$)	Long GnRH agonist protocol, fixed FSH doses after 2 weeks 225 IU/day ($n = 40$)	Proportionally less chromosomal abnormal embryos were obtained after mild ovarian stimulation

The number of included cycles is equal to the number of included patients unless stated otherwise. Outcomes were significantly different unless stated otherwise.

Storeng, 2001; Van der Auwera and D'Hooghe, 2001) as well as the chromosomal constitution of embryos (Katz-Jaffe *et al.*, 2005). Moreover, ovarian stimulation might disrupt mechanisms involved in maintaining accurate chromosome segregation (Munne *et al.*, 1997; Hodges *et al.*, 2002).

Mild stimulation approaches, aiming at a more physiological response, might therefore improve embryo quality. A randomized trial concerning the chromosomal competence of human embryos as assessed by preimplatation aneuploidy screening by fluorescent *in situ* hybridization showed a significantly higher proportion of euploid embryos following mild ovarian stimulation compared with conventional stimulation, suggesting that through maximal stimulation the surplus of obtained oocytes and embryos are of lower quality (Fig. 7) (Baart *et al.*, 2007).

A recent meta-analysis combining the results of three separate RCTs performed by our group suggests that the retrieval of a modest number of oocytes following mild stimulation is associated with a distinctly higher implantation rate compared with patients where the same number of oocytes is retrieved following conventional stimulation (Verberg *et al.*, 2008b). These observations have led to the contention that when few oocytes are obtained following mild ovarian stimulation, they are likely to represent a more homogenous group of good-quality oocytes instead of a pathological reduction in the ovarian response. These findings imply that the fear of obtaining low numbers of oocytes following mild ovarian stimulation is unjustified contradicting the assumption that an increased quantity of oocytes leads to better outcomes (Devreker *et al.*, 1999). In fact, in most of the studies investigating the relationship between oocyte numbers and pregnancy rates, the positive effect on pregnancy rates with a growing number of oocytes eventually levels off (Devreker *et al.*, 1999; Melie *et al.*, 2003; Kok *et al.*, 2006) or falls (Van der Gaast *et al.*, 2006).

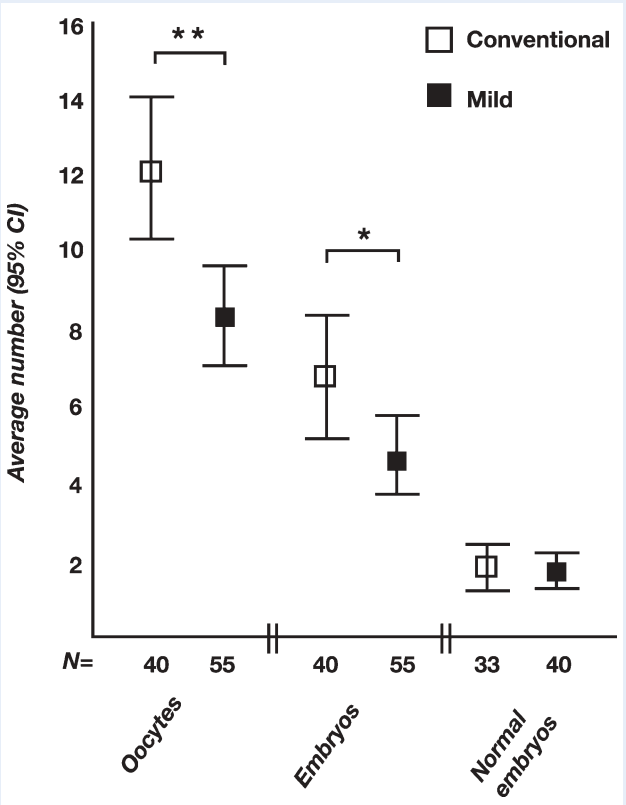

Figure 7 Oocyte and embryo yield and embryos successfully biopsied and diagnosed by fluorescent *in situ* hybridization (FISH) as chromosomally normal on the basis of FISH results form one cell following conventional and mild stimulation (Baart *et al.*, 2007).

A potential disadvantage of the development of lower numbers of oocytes might be the reduction of supernumerary embryos for cryopreservation to transfer in subsequent (unstimulated) cycles. However, as was discussed previously, the number of good-quality embryos resulting from mild ovarian stimulation was found to be similar to that following a conventional stimulation protocol and should therefore lead to a equal total number of pregnancies (Baart *et al*., 2007). Furthermore, in view of the many legal and ethical issues relating to cryopreserved embryos, the possibility of cryopreserved supernumerary oocytes rather than embryos has recently been proposed (Jain and Paulson, 2006).

Luteal function and endometrial receptivity

Ovarian stimulation affects luteal phase function and alters endometrial receptivity. This negative effect of ovarian stimulation has largely been held responsible for the impaired embryo implantation compared with natural cycles utilized in oocyte donation (Paulson *et al*., 1990). The exact pathophysiology remains unclear although supraphysiological steroid levels are widely held responsible (Beckers *et al*., 2003) (for review Fauser and Devroey 2003; Strowitzki *et al*., 2006). A negative influence of supraphysiological estradiol levels on implantation rates has been clearly established; estradiol levels >3000 pg/ml on the day of hCG administration resulted in reduced implantation rates independent of embryo quality (Simon *et al*., 1995). Mild stimulation approaches, aiming at a more physiological response, might therefore improve embryo implantation rates (Devroey *et al*., 2004). Indeed, increased pregnancy rates have been observed following a FSH step-down regimen for high response patients when estradiol levels were decreased during the preimplantation period (Simon *et al*., 1998).

Health economics considerations

Due to the limited use of ovarian stimulating medication and the decreased chances for complications such as OHSS, the per cycle cost of mild stimulation IVF will be substantially lower compared with conventional stimulation approaches. It has been calculated that the mean cost for the treatment of OHSS ranged from $400 to 553 per day depending on the treatment strategy applied, and over 6000 dollar when the cycle was cancelled (Wittenberger *et al*., 2005). However, in order to analyse the cost-effectiveness of mild stimulation, the total cost per live birth may represent the best endpoint. Besides the costs for medication, medical consultations and visits, laboratory charges (general, hormone and embryology), ultrasound procedures, IVF procedures (oocyte retrieval and embryo transfer), hospital charges, nurse coordinator costs, administrative charges, fees for anaesthesia, costs for complications, travel expenses and lost wages should all be taken into account (Collins, 2002).

Up to the present, there are few studies that (properly) analyse the cost-effectiveness of various mild stimulation approaches. Studies evaluating natural versus stimulated IVF showed that natural cycle IVF was more cost-effective than stimulated cycles per live birth (Daya *et al*., 1995; Nargund *et al*., 2001). However, it is not clear what aspects were included in these cost estimates. Cost per patient after up to three cycles of modified natural cycle IVF were found to be higher compared with a single cycle of conventional stimulation (Pelinck *et al*., 2005). In this analysis, costs of cryopreservation and OHSS were not taken into account and data for the conventional stimulation protocol were derived from the literature. CC-stimulated cycles with GnRH antagonist co-treatment were not found to be cost-effective compared with a GnRH agonist flare protocol (Kovacs *et al*., 2004) or a long GnRH agonist protocol (Mansour *et al*., 2003). However, the first study only included medication costs and although the latter included medical and treatment costs, potential additional costs were excluded.

In a prospective randomized trial regarding the efficacy of IVF using either mild ovarian stimulation in combination with SET or a long GnRH agonist co-treatment conventional stimulation protocol along with double embryo transfer, the costs and clinical outcome after 12 months of treatment were compared (Heijnen *et al*., 2007). This study showed that the overall costs resulting from treatment up until 12 months after randomization were lower for the mild strategy, despite a higher average number of IVF cycles (325 versus 444 cycles) for the mild strategy (Heijnen *et al*., 2007). However, this reduction in costs was mainly due to a reduction in multiple pregnancies and preterm births in the mild strategy (Polinder *et al*., 2008).

Psychological burden

Apart from health risks, emotional stress should be considered an important negative side effect associated with IVF treatment. The stress of infertility treatment has been ranked second to that involving the death of a family member or divorce by couples undergoing IVF treatment (Freeman *et al*., 1985; Baram *et al*., 1988). Some studies even describe an increased risk of marital stress and divorces in couples undergoing IVF treatment (Wang *et al*., 2007), although other studies have not confirmed this (Pinborg *et al*., 2003; Holter *et al*., 2006; Repokari *et al*., 2007). In contrast, findings of one study even suggested that shared stress, bereavement and disappointments can increase a couple's feeling of cohesion and result in improvement in their marriage (Repokari *et al*., 2007). The latter only appeared to be true for couples in whom treatment also results in a (singleton) live birth (Boden, 2007; Repokari *et al*., 2007).

Besides a potential direct negative effect on the chance of conceiving (Verhaak *et al*., 2001; Smeenk *et al*., 2001, 2005; Cwikel *et al*., 2004), treatment related stress was found to be the most important reason for patients dropping out of IVF treatment (Olivius *et al*., 2004). The early drop-out from treatment deprives the couple an optimal cumulative chance of achieving a pregnancy, and therefore also impacts on the overall success rates of the respective IVF programme. Average drop-out rates well above 50% have frequently been reported in the literature (Callan *et al*., 1988; Tan *et al*., 1992; Land *et al*., 1997; Olivius *et al*., 2002; Schroder *et al*., 2004).

Mild ovarian stimulation, aiming to provide a shorter and more patient-friendly treatment with a reduction in complications, might decrease IVF treatment-related stress. Following minimal intervention (unstimulated cycle or CC), patients reported fewer side effects and stress related to hormone treatment and cycle cancellation compared with conventional stimulation (Hojgaard *et al*., 2001). Furthermore, mild ovarian stimulation was found to lead to a significant reduction in drop-out rates per cycle. This observation should be considered in the context of a similar level of overall discomfort despite the increased number of treatment cycles needed to achieve a similar result as a conventional treatment group (de Klerk *et al*., 2006;

Heijnen *et al*., 2007). A mild IVF treatment strategy was found to be associated with fewer symptoms of depression after overall treatment failure than a standard IVF treatment (de Klerk *et al*., 2007). Consequently, mild stimulation might not only reduce the psychological burden of IVF treatment but it may also have a positive impact on cumulative treatment success rates as it positively affects the chance patients are willing to continue treatment following a failed attempt and therefore compensate for the lower pregnancy rate per cycle following mild stimulation (Verberg *et al*., 2008a).

Current status and future developments

Up to now, studies on alternative (milder) stimulation protocols have been limited by the relative small numbers of patients included, poor methodological quality (there are few randomized studies) and the use of surrogate end-points such as the number of oocytes or embryos. Furthermore, many studies on alternative stimulation strategies have involved poor prognosis patients, while it is mainly the young patients that have the highest risk of complications of ovarian stimulation. Therefore, especially young women should be included in studies evaluating the possible benefits of mild stimulation. Even so, most studies in older patients failed to show a benefit of a high-dose stimulation regimen over milder forms of ovarian stimulation. As most of these patients will fail to respond well to any type of ovarian stimulation, a mild stimulation protocol may be also preferred in such patients.

Increased awareness among patients and their physicians of the burden and complications associated with ovarian stimulation will facilitate the acceptance of milder stimulation (Edwards, 2007; Nargund and Frydman, 2007; Nargund *et al*., 2007; Pennings and Ombelet, 2007; Ubaldi *et al*., 2007). Crucial to the success of implementing mild ovarian stimulation strategies will be achieving a consensus as to how success and complications of IVF treatment are reported in the literature. By reporting the incidence and severity of complications, the number of treatment days, medication used, costs, patient discomfort and drop-outs, awareness of the price paid for currently applied stimulation protocols will increase. Furthermore, a reappraisal of the current paradigm of maximizing treatment outcomes per cycle at all costs is needed (Fauser *et al*., 2005). The competition for patients, desire for high fertility rates and the need for quick results driving fertility practices in some countries, are factors that could cause resistance by physicians towards the use of mild ovarian stimulation. At the end, (cumulative) mild stimulation cycles might lead to a safer and more cost-effective IVF treatment strategy.

A further prior condition for implementation of mild ovarian stimulation into standard clinical practice is the availability of (reimbursed) IVF treatment. As long as IVF is not easily accessible or patients have to pay for each cycle themselves it is likely that treatment strategies with the highest pregnancy rates (and most complications) will be preferred. On the other hand, mild IVF could be offered to patients at reduced costs. Since society will bear a large proportion of the costs associated with the complications from IVF treatment, there is a role for individual governments to assist in the uptake of mild strategies for IVF by increasing the accessibility of IVF in the public sector and encourage health insurance companies to provide full coverage of fertility treatments so that patients will be more willing to use milder strategies. It should also be emphasized that mild ovarian stimulation for IVF can only be introduced successfully in a setting with optimal laboratory performance. A reduced number of oocytes at the starting point for the IVF procedure could easily lead to too few good-quality embryos for transfer or cryostorage under suboptimal conditions, with major implications for overall success rates.

Eventually, ovarian stimulation might be replaced by *in vitro* maturation of oocytes. This technique aims at the *in vitro* culture of follicles after the retrieval of immature oocytes from unstimulated or minimally stimulated cycles. Consequently, it does not require the use of gonadotrophins for *in vivo* follicular growth and oocyte maturation (Barnes *et al*., 1995, 1996; Oktay *et al*., 1998). However, series published to date are small and even with the help of limited ovarian stimulation and hCG for oocyte maturation, pregnancy rates of 30% have only been obtained by transplanting multiple embryos, because implantation rates remain 10–15%. Insufficient data are currently available from follow-up studies to assess the safety of this technique for offspring (Chian *et al*., 1999, 2000; Rao and Tan, 2005).

Conclusions

Evidence in favour of mild ovarian stimulation for IVF is accumulating in recent literature. Namely young, good responders and polycystic ovary syndrome patients may benefit from mild stimulation. However, an important concern regarding the use of a mild treatment strategy remains the reduction in the per cycle chance of pregnancy. Again, chances for IVF success should be balanced against patient discomfort, chances for complications and costs.

Data discussed in this review do not allow any conclusions to be drawn regarding the most optimal mild ovarian stimulation protocol. Increased understanding of the physiology of follicle development has also lead to more individualized stimulation approaches (Fauser *et al*., 2008). The development of at least more than one dominant follicle appears to be necessary to produce competing IVF treatment outcomes. However, a reduction in medication is feasible when the initiation of exogenous FSH is postponed until the end of the FSH window, or when FSH is being replaced by hCG or LH when ongoing follicle growth is no longer dependent on FSH.

By suppressing endogenous negative feedback mechanisms for the natural selection of a single dominant follicle by CC or aromatase inhibitors, the use of gonadotrophins can be limited even further. A combination of these strategies might lead to an even further reduction in medication (days) needed, while it may assist in the selection of a homogenous cohort of good quality and mature oocytes and achieve a more physiological response. The implementation of mild stimulation into standard clinical practice appears to be justified, although more studies are needed to further elaborate the various mild stimulation approaches.

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