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Optimisation of the follicular phase in IVF/ICSI

C. Blockeel[1]
Promotor: P. Devroey[1]

[1]*Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium.*

Correspondence at: christophe.blockeel@uzbrussel.be

Abstract

In reproductive medicine, the aim is to establish an optimal balance between cost-effectiveness, success rate and safety for the patient. In this thesis, a series of clinical studies are presented that all revolve around the optimisation of ovarian stimulation with GnRH antagonist co-treatment. Basal hormonal levels at the start of ovarian stimulation are mandatory to obtain acceptable pregnancy rates. In view of this, we focused on the impact of cycle day 2 progesterone levels on treatment outcome. By interfering in the early follicular phase, we tried to synchronise the follicular cohort and to increase the number of retrieved oocytes.
Innovative treatment protocols based on GnRH antagonists should lead to a more flexible and better controlled schedule of oocyte retrievals. The inability to program the start of gonadotrophin stimulation and hence to minimise weekend oocyte retrievals is a major impediment to the widespread implementation of the GnRH antagonist protocol in fertility clinics. Because treatment schedule is important both for the patients, who wish to undergo reproductive treatment at their own convenience, and for the ART clinic, to organise the workload, we attempted to bring the schedule of egg retrievals in a GnRH antagonist protocol under improved control.
Another important aim of our clinical research was to diminish patient discomfort and reduce side effects by a simplification of GnRH antagonist ovarian stimulation protocols. We also focused on significant reduction of FSH consumption by substitution of FSH by low dosages of hCG during the mid-late follicular phase, without impairing outcome in terms of oocyte yield and ongoing pregnancy rate or live birth rate.

Key words: **GnRH antagonist, follicular phase, IVF, ICSI, reproductive endocrinology, scheduling.**

Introduction

The introduction of ovarian stimulation is an important milestone in the history of assisted reproductive technologies (ART), since this practice led to multiple follicular growth, the retrieval of several oocytes and consequently more embryos (Blackwell et al., 1973; Dierschke et al., 1970). Numerous stimulation regimens have been described, ranging from no stimulation (natural cycles), to minimal stimulation (clomiphene citrate) or mild stimulation (sequential treatment with clomiphene citrate and low dose exogenous gonadotrophins), to aggressive stimulation (high dose exogenous gonadotrophins, alone or in combination with a gonadotrophin-releasing hormone (GnRH) agonist or antagonist). Each approach has its advantages, disadvantages, and applications. Since the early 1980s, GnRH agonists were used for the prevention of a premature LH rise during ovarian stimulation (Fleming et al., 1982; Porter et al., 1984). Gonadotrophin-releasing hormone (GnRH) antagonists were introduced for ovarian stimulation in ART during the last decade.

Antagonists are associated with less side-effects in comparison with the GnRH agonists, because of their different mode of pharmacological action on the pituitary. The antagonistic analogue has an immediate action and thus can be administered precisely at the moment that suppression of a LH surge is needed, resulting in a shorter duration of stimulation and absence of side-effects caused by profound hypo-estrogenemia. Inadvertent administration of the GnRH analogue in early pregnancy can be avoided as the GnRH antagonist is administered in the mid-follicular phase (Tarlatzis et al., 2006). Nevertheless, GnRH antagonists offer less flexibility

regarding cycle programming compared with GnRH agonists (Tarlatzis et al., 2006).

Patients undergoing IVF/IVSI frequently experience a substantial burden and psychological distress. This burden is largely accounted for by two factors: length of treatment and side effects (Devroey et al., 2009). The patients' experience may be improved through the use of GnRH antagonists instead of GnRH agonists.

There appears to be no clinically significant difference in terms of live birth rate between GnRH antagonists and agonists: two meta-analyses comparing the two classes of GnRH analogues found almost identical odds ratios (0,82 - 0,86) for the probability of live birth, although the difference was statistically significant in one analysis (Al-Inany et al., 2006) and not in another (Kolibianakis et al., 2006). Recently, a systematic review and meta-analysis showed no difference in live birth rates between GnRH antagonists and agonists (Al-Inany et al., 2011).

Regarding secondary outcomes, both meta-analyses found shorter duration of GnRH analogue administration, decreased gonadotrophin requirements and lower incidence of ovarian hyperstimulation syndrome (OHSS) in the antagonist group. On the contrary, significantly more cumulus-oocyte complexes were retrieved in the agonist as compared with the antagonist group (Al-Inany et al., 2006; Kolibianakis et al., 2006).

More than 20 different GnRH antagonist protocols have been reported, which reflects an ongoing process of refinement and improvement (Kolibiniakis et al., 2006; Tarlatzis et al., 2006; Devroey et al., 2009).

Attempts to improve the outcome of assisted reproductive technology (ART) programs in terms of improving patient friendliness, reducing the incidence of potential complications such as cyst formation and development of the ovarian hyperstimulation syndrome (OHSS), and cutting the global cost of ART, have led to a growing interest in GnRH-antagonist protocols.

Basal hormonal levels at the start of ovarian stimulation are mandatory to obtain acceptable pregnancy rates. Elevated progesterone levels at the start of ovarian stimulation in patients treated with rFSH and GnRH antagonists are associated with reduced pregnancy rates (Kolibianakis et al., 2004). However, the incidence of this condition has been poorly documented. The purpose of our cohort study was to prospectively compare the ongoing pregnancy rates in patients with normal and elevated progesterone levels on day 2 of the treatment cycle in a GnRH antagonist protocol (Blockeel et al., 2011b).

In line with this trial, we subsequently hypothesised that pretreatment with GnRH antagonists could be proposed as a planning tool for follicular cohort synchrony and scheduling of ART treatment in all patients, including those with normal serum progesterone levels on day 2 of the treatment cycle. The purpose of this next prospective randomised trial was to study the impact of a three-day course of GnRH antagonist pretreatment on the number of COCs (Blockeel et al., 2011c).

Apart from the aim to improve pregnancy rates, innovative treatment protocols based on GnRH antagonists should lead to a more flexible and better controlled schedule of oocyte retrievals, such as in the long GnRH agonist protocol. The inability to program the start of gonadotrophin stimulation and hence to minimise weekend oocyte retrievals is a major impediment to the widespread implementation of the GnRH antagonist protocol in fertility clinics. Because treatment schedule is important both for the patients, who wish to undergo reproductive treatment at their own convenience, and for the ART clinic, to organise the workload, we attempted to bring the schedule of egg retrievals in a GnRH antagonist protocol under improved control. Oral contraceptive pill (OCP) pretreatment is increasingly being abandoned as a planning tool, since this results in significantly lower pregnancy rates (Griesinger et al., 2010). It has been hypothesised that the gestagen component of the OCP could exert a negative impact on endometrial receptivity in the subsequent cycle. Alternatively, low endogenous LH levels after OCP pretreatment might impair oocyte competence or endometrial receptivity when ovarian stimulation is performed with recombinant FSH without LH in GnRH antagonist cycles (Griesinger et al., 2008).

The aim of this study was to prospectively analyse the ability to control the scheduling of GnRH antagonist cycles by the administration of estradiol valerate during the luteo-follicular transition period prior to the initiation of ovarian stimulation (Blockeel et al., 2012, RBMOnline).

Scarce information is currently available regarding the endocrine profile of the follicular phase during the so-called 'mild ovarian stimulation' protocols (Hohmann et al., 2003). We conducted a randomised, controlled trial to compare endocrine parameters and follicular development in IVF/ICSI cycles during which stimulation was started either on cycle day 2 or 5, and to evaluate the clinical applicability of a deferred start of gonadotrophin stimulation. The hypothesis was that when the start of gonadotrophin is postponed until cycle day 5, combined with an early start of the GnRH antagonist, a mild ovarian response would occur, characterised by a moderate estradiol rise, whilst preserving LH suppression (Blockeel et al., 2011d).

For many years, FSH was considered the only stimulatory hormone needed for ovulation induction, acting through specific receptors on granulosa cells of ovarian follicles. In a GnRH agonist protocol, it was demonstrated that the administration of low doses of hCG (100-400 IU/day) can be successfully applied in patients undergoing ART as a substitute for recombinant FSH in the final days of ovarian stimulation (Filicori et al., 2002a). In GnRH antagonist cycles, mid-late follicular phase FSH substitution by hCG, rather than supplementation, has been reported in a small cohort study, but this approach has not been investigated in a randomised controlled fashion (Kenigsberg et al., 2006). Taking into account the more profound suppression of endogenous LH-levels by a GnRH-antagonist as compared to a GnRH-agonist, we conducted a preliminary study to explore whether low doses of hCG (200 IU/d) have similar potential to stimulate follicular development and yield satisfactory ongoing pregnancy rates in the absence of FSH (Blockeel et al., 2009). As a continuation of this trial, we aimed to assess the influence of the administration of low dose hCG in the late follicular phase before final oocyte maturation on endometrium histology; we studied the morphological pattern and gene expression profile of human endometrium on the day of oocyte retrieval (Blockeel et al., 2011a).

Methods

All the studies were conducted in the period between 2007-2011 at the Centre of Reproductive Medicine of UZ Brussel. All patients with primary or secondary infertility were included. A complete hormonal investigation with FSH, LH, E_2, testosterone, delta4-androstendione, prolactine, DHEAS, TSH, T3 and T4 was performed. A complete serological screening was added (Hepatitis B, C, HIV, Syphilis, CMV, Toxoplasmosis and Rubella) before being included. Before entering the study, all patients underwent a complete pelvic examination and an ultrasound scan. Ethical approval was obtained by the Ethical Committee of the Universitair Ziekenhuis Brussel. In case of a RCT, randomisation was performed at the outpatient clinic, when the results of the pre-treatment hormonal analyses were discussed with the patient. A computer-generated list was used for randomisation, concealed to the recruiting nurse who made the decision about allocation by using a series of consecutively numbered sealed opaque envelopes. The treating physicians remained blind to the intervention group of each patient throughout the ovarian stimulation cycle, but the study nurses and the patients were aware of treatment group allocation. Each patient gave written informed consent and was enrolled in a study only once.

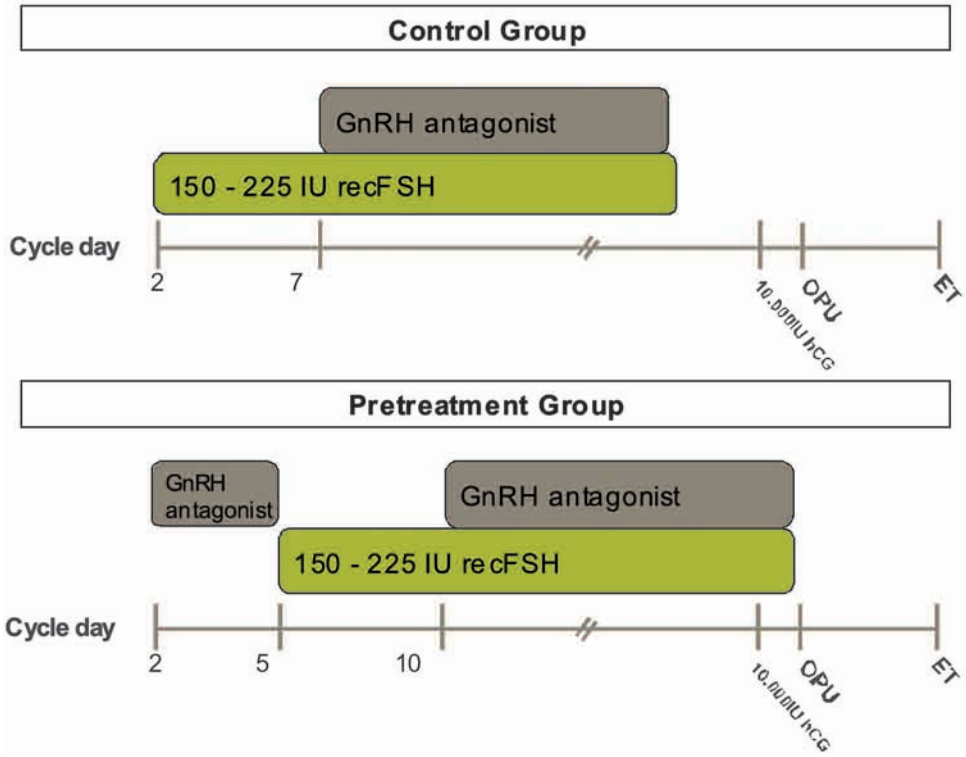

Fig. 1. — Randomised controlled trial with GnRH antagonist pretreatment in the study group (Blockeel et al., 2011)

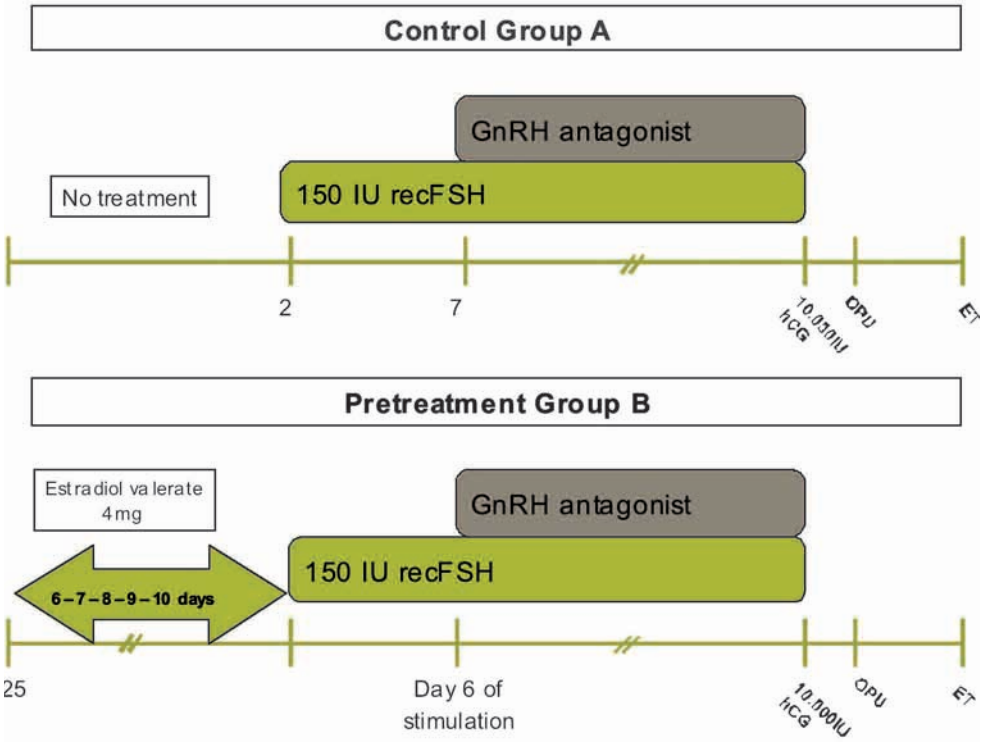

Fig. 2. — Randomised controlled trial with estradiol valerate pretreatment in the study group (Blockeel et al., 2012)

Multifollicular ovarian stimulation

Since protocols for ovarian stimulation differ significantly in the presented studies, the methodology is described in Figures 1 to 4. Final oocyte maturation was triggered by the administration of 10,000 IU hCG (Pregnyl®, MSD, Oss, The Netherlands), as soon as three follicles of 17 mm diameter were visualised on ultrasonography (Kolibianakis et al., 2004). Cumulus-oocyte-complexes (COC) were

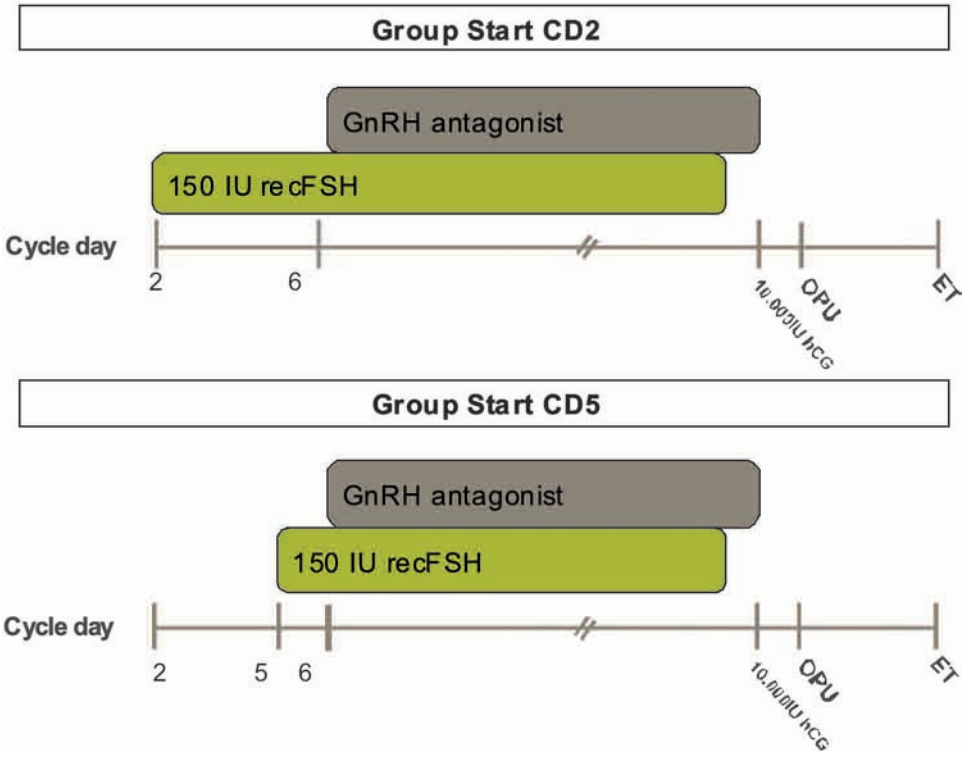

Fig. 3. — Randomised controlled trial with start of stimulation on cycle day 5 in the study group (Blockeel et al., 2011)

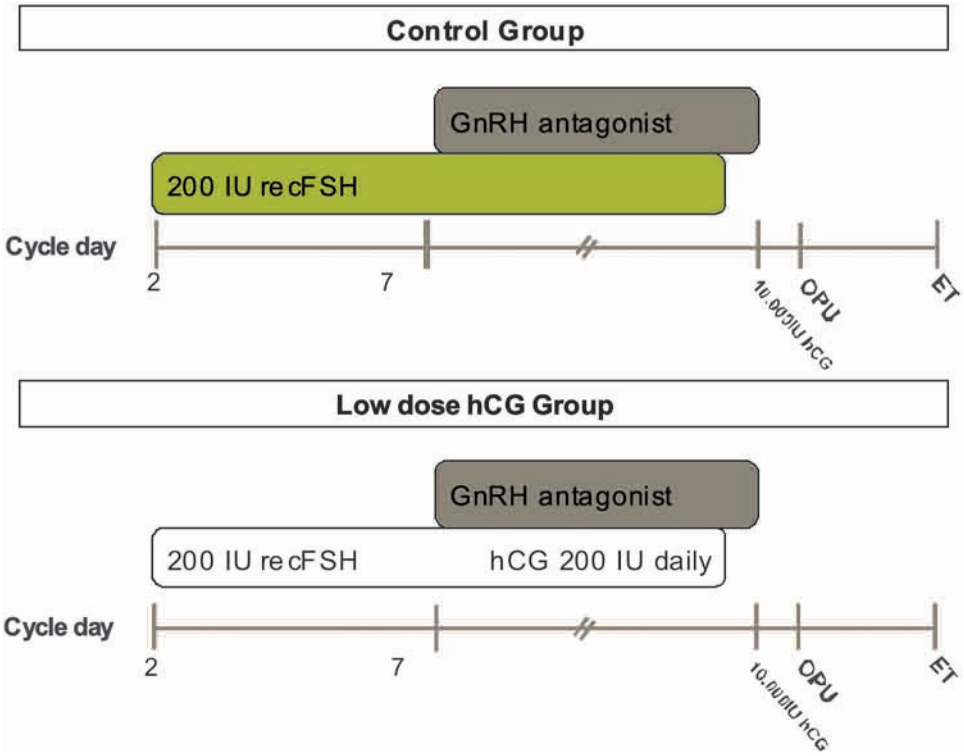

Fig. 4. — Randomised controlled trial with low dose hCG as a substitution of FSH in the late follicular phase (Blockeel et al., 2009)

collected 36 hours after Pregnyl® administration. Luteal phase support consisted of 600 mg of vaginally administered micronised natural progesterone (Utrogestan®, Besins International, Paris, France) per day. To assess the treatment outcome, serum hCG was measured 14 and 17 days after oocyte retrieval. HCG levels above 20 IU/l were indicative for occurrence of a pregnancy.

Embryo culture, evaluation and embryo transfer

In the trials, conventional IVF or ICSI was performed. Procedures for intracytoplasmic sperm injection were carried out as described by Van Landuyt et al. (2005). Normal fertilisation was checked on day 1. Embryo quality was assessed daily from day 2 onwards until the moment of transfer or cryopreservation (in case of good-quality spare embryos), as described by Papanikolaou et al. (2005a,b). The embryo quality on day 5 was assessed according to the criteria of Gardner and Schoolcraft (1999). All transfers were single embryo transfers on day 5, except for the prospective cohort trial (Blockeel et al., 2011b) and the D2 versus D5 trial, where day 3 embryo transfer was also allowed.

Results

GnRH antagonist pretreatment in case of elevated progesterone

A total of 484 patients participated in this prospective cohort study. Abnormal progesterone levels on day 2 of the cycle were recorded in 30 out of 484 patients (6.2%). Subsequent administration of a GnRH antagonist on three consecutive days in the high progesterone group resulted in the normalization of progesterone values in all patients (Fig. 1).

Serum hormone levels of FSH, LH, E_2 and progesterone on the day of hCG trigger administration were similar in both treatment groups. The pregnancy rates per started cycle, per oocyte pick-up and per embryo transfer were not significantly different in both treatment groups (Table I).

GnRH antagonist pretreatment in case of normal progesterone

A total of 69 patients were randomly assigned to either the control group (n = 36) or the pretreatment group (n = 33). The groups did not significantly dif-

Table I. — GnRH antagonist pretreatment in case of elevated progesterone: clinical outcome measures (Blockeel et al., 2011b).

	Normal P (P < 1.5 ng/mL)	Elevated P (P > 1.5 ng/mL)	p value
Positive hCG			
Per started cycle % (n)	31.9 (145/454)	23.3 (7/30)	0.33
Per pickup % (n)	32.1 (145/452)	26.9 (7/26)	0.58
Per embryo transfer % (n)	36.1 (145/402)	29.2 (7/24)	0.49
Outcome for patients with positive hCG test			
Biochemical pregnancy % (n)	10.3 (15/145)	42.9 (3/7)	0.01
Miscarriage % (n)	9.7 (14/145)	0.0 (0/7)	0.38
Ectopic pregnancy % (n)	2.8 (4/145)	0.0 (0/7)	0.66
Ongoing pregnancy % (n)	77.2 (112/145)	57.1 (4/7)	0.22

fer with regard to baseline and demographic characteristics, such as age, BMI, basal FSH, antral follicle count, number of previous IVF/ICSI trials and indication for infertility treatment.

The duration of hormonal stimulation (8.8 vs 8.9 d) and total dose of gonadotropins (1568.2 IU vs 1499.6 IU) were similar in the two groups. The number of COCs retrieved was 9.9 (SD 4.9) and 12.8 (SD 7.8) in the control and pretreatment group, respectively, with a between group difference of 2.9 and 95% confidence limits of -0.2 to 6.0 (p = 0.067). Among the patients who had oocyte retrieval (36 control vs 31 women in the pretreatment group) the crude and adjusted between-group differences for the number of COCs retrieved were 3.7 (0.7 to 6.7; p = 0.016) and 3.9 (0.9 to 7.0; p = 0.012), respectively. Ongoing pregnancy rates per started cycle, per oocyte retrieval and per embryo transfer were similar in both groups (Table II).

Estradiol valerate pretreatment

A total of 86 patients were randomly assigned to either the control group or the pretreatment group (Fig. 2). Demographic characteristics did not differ significantly between both groups.

With regard to the outcome of ovarian stimulation in both groups, a longer duration of stimulation was observed in the pretreatment group [9.6 (SD 1.4) days versus 8.6 (SD 1.5) days in the control group (p = 0.004)]. The number of COCs obtained at retrieval was similar in both groups. The proportion of patients undergoing an oocyte retrieval during the weekend was significantly lower in the pretreatment group (1/37) compared to the control group (8/39), with an absolute between-group difference of -17.8% (95% confidence interval -31.5% to -4.1%, p = 0.029). Only one oocyte retrieval occurred on a Saturday in the pretreatment group (Fig. 5).

The ongoing pregnancy rates per started cycle were similar in the pretreatment group (16/42 or 38.1%) and the control group (16/44 or 36.4%), between-group difference -1.7%, p = 0.868).

Start of ovarian stimulation on day 2 or day 5 of the cycle

Overall duration of rFSH administration (days of stimulation) in the study group was significantly lower than in the control group. The total dose of rFSH consumed was significantly lower in the study group: 1,364 IU (SD 226) versus 1,177 IU (SD 295),

Table II. — GnRH antagonist pretreatment in case of normal progesterone (Blockeel et al., 2011c).

	Control group	Pretreatment group	P - value
Starting dose of rFSH (IU)	177.7 ± 32.3	166.9 ± 22.9	0.125
Days of rFSH stimulation	8.8 ± 1.7	8.8 ± 1.4	1.000
Number of COCs*	9.9 ± 4.9	13.6 ± 7.3	**0.016**
Ongoing pregnancy rate per started cycle % (n)	33.3% (12/36)	42.4% (14/33)	0.596

* Number of COCs among patients who underwent oocyte retrieval.

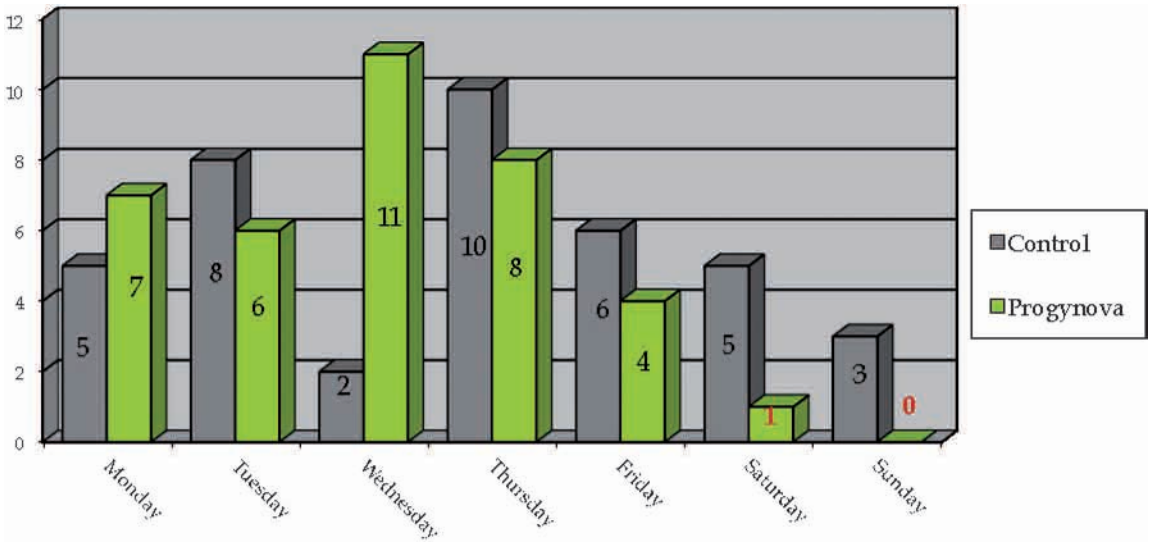

Fig. 5. — Oocyte retrieval by day in the estradiol valerate pretreatment group and the control group (Blockeel et al., 2012)

$p < 0.01$. The number of COCs obtained at retrieval was similar in both groups. The fertilisation rate, the implantation rate, the pregnancy rate and abortion rate were comparable in both groups. The ongoing pregnancy rate per started cycle was 28% (10 out of 36 patients in the control group and 25% (10 out of 40 patients) in the study group ($P = 0.78$). Serum hormone levels were measured on day 2, day 6 and day 8 of the cycle, and immediately before triggering final oocyte maturation. Follicular phase patterns of both gonadotropin and steroid levels did not demonstrate clear differences between both treatment groups, except for the serum estradiol and FSH level being significantly higher and the serum LH level being significantly lower on day 6 of the cycle in the control group. Also, LH levels appeared significantly higher in the study group at the time of hCG.

Low dose hCG in the late follicular phase

A total of 70 patients were randomly assigned to either the study or the control group (Fig. 5). Overall treatment duration (days of stimulation) did not significantly differ. Obviously, the duration of rFSH administration in the hCG-group was significantly lower than in the control group. HCG was administered in the hCG-group for 2.3 ± 1.4 days. The total dose of rFSH consumed was significantly lower in the hCG group: 1273 (SD 260) versus 1617(SD 280), $p < 0.001$. The number of COCs obtained at retrieval was similar in both groups. The fertilisation rate, the implantation rate, the pregnancy rate and abortion rate were comparable in both groups (Table III).

Influence on the endometrium of low dose hCG in the late follicular phase

A total of 35 patients were randomly assigned to either the study or the control group. All patients underwent an endometrial biopsy on the day of oocyte retrieval. There was no difference in both groups in terms of histological dating of the endometrium on the day of oocyte retrieval. Gene expression analysis was performed on 5 non-pregnant patients from the hCG-group and 5 non-pregnant patients from the control group. It showed a significant differential expression of 65 probe sets between the hCG- and the rFSH-group, with 21 upregulated and 44 downregulated in the hCG-group. Unsupervised principal component analysis (PCA) with GeneSpring GX 7.3 was applied to reduce the multidimensional gene expression data into three dimensions. It showed a heterogenous expression pattern with no specific clusters for both study groups.

Discussion

A key step forward in developing strategies for IVF stimulation was the introduction of GnRH antagonists. In contrast to GnRH agonists that rely on pituitary desensitisation, GnRH antagonists cause immediate gonadotrophin suppression by competitive occupancy of the GnRH receptor. As stated in the introduction, GnRH antagonist regimens yield shorter stimulation cycles, resulting in fewer injections and therefore a substantial decrease of the patients' burden (Devroey et al., 2009). Moreover, side-effects and risk of OHSS and cycle cancellation

Table III. — Low dose hCG in the late follicular phase: cycle outcome measures (Blockeel et al., 2009).

	rFSH-group Mean (SD)	Low-dose hCG group Mean (SD)	P value for between-group difference
Total dose of rFSH, IU	1617 (280)	1273 (260)	**< 0.001**
Overall treatment duration, days	8.2 (1.6)	8.7 (1.6)	0.19
Rec FSH duration, days	8.2 (1.6)	6.4 (1.3)	**< 0.001**
hCG duration, days	0	2.3 (1.4)	**< 0.001**
Number of COCs	12.3 (5.8)	11.1 (5.2)	0.40
Number of ongoing pregnancies n(%)			
Per started cycle	10 / 35 (29%)	13 / 35 (37%)	0.45
Per pickup	10 / 32 (31%)	13 / 29 (45%)	0.28
Per embryo transfer	10 / 29 (35%)	13 / 27 (48%)	0.30

are reduced in antagonist cycles (Kolibianakis et al., 2006; Heijnen et al., 2007; Al-Inany et al., 2011).

In the work presented in this thesis, I have aimed to define new strategies for ART cycles, stimulated with GnRH antagonist co-treatment. The focus of these strategies was to improve the balance between treatment efficacy and burden. In the first part of the work, I focused on the luteo-follicular transition phase and early follicular phase, whereas in the second part, I tried to optimise the mid-late follicular phase.

Early Follicular Phase

Progesterone levels at the start of ovarian stimulation in patients treated with rFSH and GnRH antagonists are of crucial importance. Although the occurrence has been poorly documented, elevated progesterone levels on day 2 or 3 of the cycle are associated with reduced pregnancy rates (Kolibianakis et al., 2004). In this trial, we demonstrated that raised progesterone levels can be normalised through administration of a GnRH antagonist during three subsequent days prior to the start of gonadotropin stimulation.

In case of normal progesterone at initiation of the cycle, pretreatment with GnRH antagonists during three consecutive days before the onset of ovarian stimulation yields a higher number of COCs per oocyte retrieval compared to a conventional GnRH antagonist protocol. This approach could be adopted as novel convenience tool to schedule GnRH antagonist cycles and to aid the organisation of an ART centre, through the administration of a GnRH antagonist during a varying number of days (i.e. two, three or four days), in accordance with the intended onset of ovarian stimulation. The protocol could be used in oocyte donors to facilitate synchronisation with the acceptor. The clinical potential of this short and patient-friendly modified protocol is associated with a small financial cost increment of approximately 110 euros per cycle. Another possible drawback of this pretreatment protocol is the addition of three subcutaneous injections to the patient.

Pretreatment with estradiol valerate reduces the proportion of oocyte retrievals during weekend days, without affecting the number of COCs or ongoing pregnancy rates. The clinical potential of this short and patient-friendly protocol is associated with a smaller financial cost increment than the GnRH antagonist pretreatment protocol, besides the absence of supplementary injections in the estradiol pretreatment protocol. Using this protocol, we are able to schedule the oocyte retrievals without changing the criteria for final oocyte maturation, as prolongation of the follicular phase by delaying the administration of hCG is shown to result in lower pregnancy rates (Kolibianakis et al., 2004).

The administration of rFSH starting on day 2 or 5 of the cycle in a GnRH antagonist co-treatment protocol for IVF/ICSI patients, yields only subtle differences in the endocrine profile and follicular development. Ovarian stimulation is initiated 3 days later in the CD5 study group compared to the CD2 control group, which implies a significant longer follicular phase in the CD5 group (10.1 days in the CD2 group versus 11.9 days in the CD5 group, $p < 0.01$). The duration of the stimulation differs by 1.3 days only. This finding suggests indeed that less administered FSH is needed when starting with stimulation later during the follicular phase and a significant decrease in total consumption of rFSH (1,364 ± 226 (SD) IU versus 1,177 ± 295 IU; $p < 0.01$). More specifically, the need for exogenous FSH during the first days of ovarian stimulation can be questioned, as the relatively high endogenous FSH concentrations during this period could be sufficient. Although

the administration of rFSH on day 5 of the cycle can overrule single dominant follicle selection in the majority of women, more than ten per cent of the patients (4 out of 39) developed mono-follicular growth, probably due to closure of the FSH window (Fauser et al., 1993). Ovarian stimulation seems to be feasible in the majority of patients when initiated on cycle day 5, with minimal impact on the length of the follicular phase. These results suggest that by selecting the appropriate day to initiate ovarian stimulation (namely on cycle day 2, 3, 4 or 5) oocyte retrievals on weekends can be largely avoided and could serve as a additional planning tool to schedule IVF treatment cycles at the patient's and the centre's convenience. The development of a standardised protocol most suitable for ovarian stimulation for all women seems to be elusive due to important differences in ovarian response among patients. Patient tailored regimens based on individual patient characteristics should be developed further. In other words, mild response rather than mild stimulation needs further scrutiny (Fauser et al., 2010).

Late Follicular Phase

The administration of 200 IU/d of hCG can be safely applied in patients undergoing ART to substitute for recombinant FSH in the final days of ovarian stimulation in an antagonist protocol. The protocol is generally applicable and the use of hCG confers a dramatic reduction on the treatment cost, as compared to FSH-containing preparations, like recombinant FSH or highly purified human menopausal gonadotropins or hMG.

Substitution of FSH by hCG has been reported earlier in GnRH agonist protocols (Filicori et al., 2002a, 2002b, 2005).

Besides the clinical aspects, the impact on endometrial gene expression in a standard rFSH stimulation protocol, compared with a 200IU hCG regimen in the late follicular phase in a GnRH antagonist protocol was assessed. No difference in terms of histological dating of the endometrium on the day of oocyte retrieval could be demonstrated, and full human genome gene expression analysis showed a significant differential expression of 65 probe sets only.

Although still to be elucidated, the expression of the LH/hCG receptor could constitute as a marker of endometrial receptivity. It is increasingly clear that hCG in the luteal phase intervenes in the regulation of endometrial differentiation with a positive impact at the different steps of implantation, tissue remodeling and angiogenesis (Perrier d'Hauterive et al., 2007). From previous studies, it was also suggested that hCG in the follicular phase positively influences implantation rates (Filicori et al., 2005). As a corollary of this, the introduction of hCG in the late follicular phase could enhance uterine receptivity and therefore play a significant role in implantation.

Conclusion

In this thesis, attempts to further optimise the GnRH antagonist protocol in order to increase the acceptance and the implementation of GnRH antagonists in general practice have been made. From a first trial, we concluded that administration of a GnRH antagonist normalises progesterone levels in those cases with isolated elevated serum progesterone levels at the start of the ART treatment cycle, and that this pretreatment is compatible with adequate ovarian stimulation and results in acceptable pregnancy rates. In the following trial, pretreatment with GnRH antagonists in case of normal progesterone yielded a trend towards a higher number of COCs compared to a conventional GnRH antagonist protocol. The next trial, making use of estradiol valerate as pretreatment, showed a reduction of the proportion of oocyte retrievals during weekend days, without affecting the number of COCs or ongoing pregnancy rates. The fourth study shows that the administration of rFSH starting on day 2 or day 5 of the cycle in a GnRH antagonist protocol for IVF/ICSI patients yields a comparable endocrine profile and follicular development. The fifth and sixth study dealt with replacing rFSH by 200IU of hCG per day in the late follicular phase of a GnRH antagonist protocol, resulting in a decreased consumption of gonadotrophins (without differences in oocyte yield and pregnancy outcome) and a similar endometrial histological and gene expression pattern.

In conclusion, the purposes of the different studies, namely increasing pregnancy chance, offering a scheduling tool for the GnRH antagonist protocol and significantly reducing the treatment cost were reached.

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