Reproductive BioMedicine Online (2012) **24**, 272–280

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ARTICLE

Oestradiol valerate pretreatment in GnRH-antagonist cycles: a randomized controlled trial

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Abstract This randomized controlled trial analyses the ability to control the oocyte retrieval schedule of gonadotrophin-releasing hormone antagonist cycles through the administration of oestradiol valerate during the luteo-follicular transition period prior to the initiation of ovarian stimulation. Eighty-six women undergoing ovarian stimulation for IVF/intracytoplasmic sperm injection were enrolled in the study. The control group ($n = 42$) received a standard ovarian stimulation protocol. In the pretreatment group ($n = 44$), patients were administered oestradiol valerate at a daily dose of 2×2 mg from day 25 of the preceding cycle onwards, during 6–10 consecutive days, depending on the day of the week. The primary endpoint was the proportion of patients undergoing oocyte retrieval during a weekend day (i.e. Saturday or Sunday), which was significantly lower in the pretreatment group (1/37, 2.7%) compared with the control group (8/39, 20.5%; P value = 0.029). The clinical pregnancy rates per started cycle were similar in the pretreatment group (38.6%) compared with the control group (38.1%). Pretreatment with oestradiol valerate results in a significantly lower proportion of patients undergoing oocyte retrieval during a weekend day and can be a valuable tool for the organization of an assisted reproduction centre.

KEYWORDS: GnRH antagonist, ICSI, oestradiol valerate pretreatment, ovarian stimulation, scheduling, reproductive endocrinology

Introduction

Gonadotrophin-releasing hormone (GnRH) antagonists have emerged in recent years in ovarian stimulation protocols for assisted reproductive treatment to inhibit an endogenous surge of LH. The advantages of ovarian stimulation with GnRH-antagonist over GnRH-agonist co-treatment are well established. Firstly, antagonists cause immediate and reversible suppression of gonadotrophin production, which results in a shorter treatment period and in less treatment burden and patient distress (Al-Inany et al., 2006; Devroey et al., 2009; European and Middle East Orgalutran Study

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2011.11.012

Group, 2001). Secondly, the use of GnRH antagonists is associated with a significantly lower probability of hospital admission due to ovarian hyperstimulation syndrome (OHSS) (Kolibianakis et al., 2006). Moreover, in patients who undergo ovarian stimulation with GnRH antagonists, the risk of OHSS can be almost completely eliminated when a GnRH-agonist trigger instead of human chorionic gonadotrophin (HCG) is used to induce final oocyte maturation, without adversely affecting implantation and pregnancy rates (Gómez et al., 2010; Humaidan et al., 2011). Finally, there appears to be no evidence of a difference between GnRH antagonists and agonists in terms of live-birth rate (Al-Inany et al., 2011).

In spite of the clear benefits associated with GnRH antagonists, GnRH agonists remain the GnRH analogue of choice in the majority of assisted reproduction clinics. The flexibility of the long GnRH-agonist protocol allows a more flexible and better controlled schedule of oocyte retrievals, whereas the initiation of ovarian stimulation in GnRH-antagonist cycles depends on the random occurrence of spontaneous menses (Tarlatzis et al., 2006). Therefore, the inability to programme the start of gonadotrophin stimulation, and hence to minimize weekend oocyte retrievals, is a major impediment to the widespread implementation of the GnRH-antagonist protocol in fertility clinics. Because the treatment schedule is important both for the patient, who wants to undergo reproductive treatment at their own convenience, and for the assisted reproduction clinic, which needs to organize the workload, several attempts have been made to bring the schedule of egg retrievals in a GnRH-antagonist protocol under better control. The use of oral contraceptive pills (OCP) has been advocated as a programming method for IVF cycles using GnRH antagonists (Fischl et al., 2001; Meldrum et al., 2002) but there is published evidence that this method yields significantly lower pregnancy rates (Griesinger et al., 2008, 2010).

Another tool to schedule the egg retrievals is delaying the administration of HCG. However, in a randomized controlled trial of 413 patients undergoing ovarian stimulation with GnRH-antagonist co-treatment, prolongation of the follicular phase for 2 days after the point at which at least three follicles were $\geq$17 mm resulted in a lower probability of ongoing pregnancy per oocyte retrieval and per embryo transfer, compared with no delay (Kolibianakis et al., 2004). This difference was probably due to secretory changes in the endometrium and could not be attributed to differences in the number and quality of the embryos transferred (Kolibianakis et al., 2005).

In contrast with these findings, a retrospective trial by Tremellen and Lane (2010) demonstrated that the advancement or delay of HCG administration by 1 day had no adverse effect on IVF live-birth success. Hence, it would be safe to advance an ideal Saturday oocyte retrieval to Friday and to delay an ideal Sunday oocyte retrieval to Monday.

De Ziegler et al. (1999) examined the feasibility of scheduling the ovulation in women (with a menstrual cycle that varies between 25 and 35 days) by administration of exogenous oestradiol at doses that duplicate circulating oestradiol luteal-phase concentrations. The intercycle FSH rise was initiated by discontinuing the oestradiol valerate treatment that had been started in the luteal phase. It is also feasible, practical and not counterproductive to use exogenous oestradiol for the control of the timing of the early follicular-phase rise of FSH; this method would create the opportunity of programming the onset of ovarian stimulation (de Ziegler et al., 1998). Furthermore, luteal oestradiol pretreatment also promotes follicular growth co-ordination during ovarian stimulation (Fanchin et al., 2005).

In 2010, a randomized controlled trial (Guivarc'h-Levêque et al., 2010) comparing 412 long agonist cycles with 426 antagonist cycles with an oestradiol valerate pretreatment demonstrated a lower proportion of oocyte retrievals during the weekend (Saturday or Sunday) without affecting pregnancy rates.

The aim of the present study was to prospectively evaluate the efficacy of scheduling assisted reproduction cycles in a GnRH-antagonist protocol through the flexible pretreatment with oestradiol valerate during the luteo-follicular transition period of the menstrual cycle.

Materials and methods

Study design

This randomized controlled trial was conducted in 86 normogonadotrophic women enrolled in an assisted reproduction programme between May 2010 and May 2011. In the control group, a standard GnRH-antagonist protocol was applied; the pretreatment group underwent a modified treatment protocol with oestradiol valerate administration during 6–10 consecutive days (from cycle day 25 onwards) prior to the start of recombinant FSH (rFSH) stimulation, so that the first day of stimulation occurs between a Friday to Sunday. Randomization was performed at the outpatient clinic on day 21 of the previous cycle. A computer-generated list was used for randomization, concealed to the recruiting nurse who made the decision about the allocation by using a series of consecutively numbered sealed opaque envelopes. Each patient was enrolled into the study only once. Patients gave written informed consent. The study was registered with ClinicalTrials.gov (number NCT01218386) and the study received institutional review board approval by the local institute's Ethics Committee (IRB reference 2010/083).

The patients included in the trial were women $\leq$36 years of age at the time of randomization, with body mass index 18–29 kg/m^2 (both inclusive), who underwent a first or second treatment cycle of IVF with intracytoplasmic sperm injection (ICSI), with a serum FSH concentration on day 3 of the menstrual cycle below 12 IU/l, a normal ultrasound scan, i.e. presence of both ovaries, without evidence of abnormality within 6 months prior to randomization and a regular menstrual cycle of 21–35 days, presumed to be ovulatory. Only ICSI cycles were included in order to avoid confounding factors and to enable access to the oocyte maturation rate.

Oocyte donors were excluded from the study, as well as patients with endometriosis grade 3 or more (according to the American Fertility Society classification of endometriosis), endocrine or metabolic abnormalities, polycystic ovary syndrome or a previous history of poor ovarian response, defined as development of $<$4 follicles in a previous IVF or ICSI cycle.

Multifollicular development

A first blood sample analysis was performed on day 21 of the menstrual cycle in both groups.

In both treatment groups, a GnRH-antagonist protocol with rFSH was applied. Daily injections of rFSH (Puregon; MSD, Oss, The Netherlands) were initiated at a dose of 150 IU/day on day 2 of the menstrual cycle in the control group and 1 day after discontinuation of the oestradiol in the pretreatment group. On day 6 of stimulation, subcutaneous administration of the GnRH antagonist ganirelix (Orgalutran; MSD) was started at a daily dose of 0.25 mg. From day 6 of the stimulation onwards, ovarian ultrasound scans to assess follicular growth and blood sampling for oestradiol, progesterone, FSH and LH concentrations were performed every other day, to adjust the dose of rFSH if necessary. The dose of rFSH remained constant during ovarian stimulation unless there was no increase in serum oestradiol concentrations after 5 days of stimulation. In the pretreatment group, oestradiol valerate (Progynova; BayerScheringPharma, Antwerp, Belgium) at a daily dose of 2×2 mg (2 mg in the morning, 2 mg in the evening) was administered orally from day 25 of the menstrual cycle onwards, during 6–10 consecutive days, depending on the day of the week that the oestradiol valerate had been started (for 6 days if day 25 was Monday, for 10 days if Tuesday, for 9 days if Wednesday, for 8 days if Thursday, for 7 days if Friday, for 6 days if Saturday and for 6 days if Sunday). This scheduling was chosen to attempt to spread the oocyte retrievals over the five week days. An outline of both treatment groups is presented in **Figure 1**.

Final oocyte maturation was triggered by the administration of 10,000 IU HCG (Pregnyl; MSD), when three follicles of 17 mm diameter were observed on ultrasound scan. Cumulus–oocyte–complexes (COC) were collected 36 h after Pregnyl administration. Luteal-phase support consisted of 600 mg of vaginally administered micronized natural progesterone (Utrogestan, Besins International, Paris, France) per day. In case of risk for OHSS, final oocyte maturation was triggered with a single dose of GnRH agonist (Decapeptyl 0.2 mg) instead of HCG, followed by a single injection of HCG 1500 IU 1 h after the egg retrieval and oestradiol valerate was orally administered in the luteal phase, in addition to progesterone.

To assess the treatment outcome, serum HCG was measured 14 and 17 days after oocyte retrieval. HCG concentrations above 20 IU/l indicated pregnancy. Clinical pregnancy was defined by the observation of fetal cardiac activity on ultrasound scan at 7 weeks of gestation.

Embryo culture, evaluation and embryo transfer

Procedures for ICSI were carried out as described by Van Landuyt et al. (2005). Normal fertilization was checked on day 1. Embryo quality was assessed daily from day 2 onwards until the moment of transfer or cryopreservation, as described by Papanikolaou et al. (2005a,b). The embryo quality was assessed according to the criteria of Gardner and Schoolcraft (1999). All transfers were single-embryo transfers on day 5.

Outcome measures

The primary endpoint was the number of patients undergoing oocyte retrieval during weekend days (i.e. Saturday or Sunday). Secondary endpoints included the mean number of COC in each treatment group, the number of metaphase-II oocytes and 2-pronuclei oocytes, the duration of stimulation and total cumulative dose of rFSH used, the pregnancy rate in each treatment group and the basal hormonal serum values. Demographic and clinical

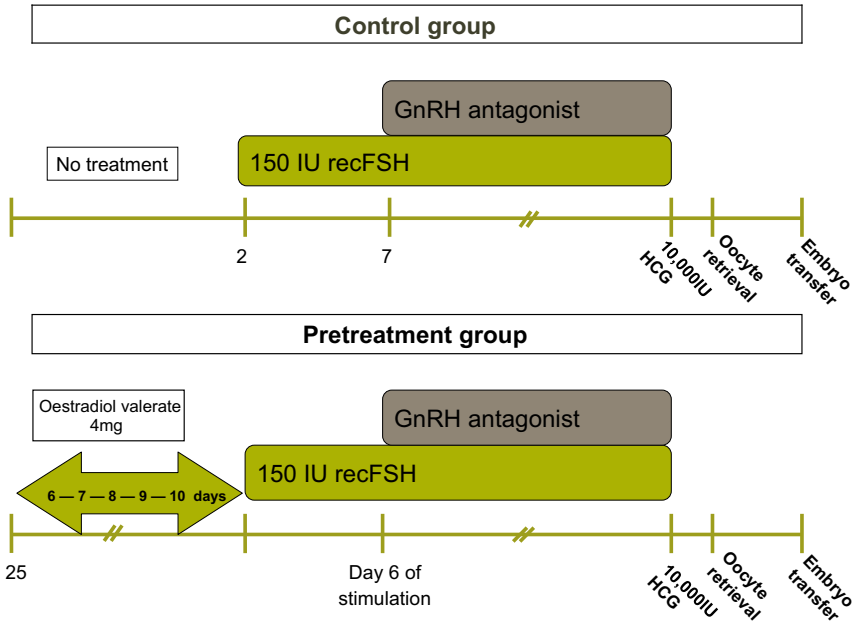

Figure 1 Schematic overview of both treatment groups. GnRH = gonadotrophin-releasing hormone; HCG = human chorionic gonadotrophin.

characteristics, such as age, weight and height were also collected.

Sample size calculation

For the sample size calculation, this study anticipated a proportion of 28.6% oocyte retrievals to occur during a weekend day (i.e. on 2 out of 7 days) in the control group and considered a decrease to 5% of oocyte retrievals occurring during the weekend days to be clinically relevant. A drop-out rate of 10% was also expected. With a type 1 error (alpha) of 5% and power (1 minus beta) of 80%, sample size calculation revealed that 84 patients in total were required to demonstrate this decrease from 28.6% to 5% oocyte retrievals occurring on a weekend day.

Statistical analysis

Data are presented as number of cases including numerator and denominator values (and percentages) for categorical variables and as mean values and standard deviation for continuous variables. Differences between treatment arms are presented as absolute between-group differences, with corresponding 95% confidence intervals and *P* values for each comparison made. Differences between treatment arms were assessed using the t-test for independent samples for continuous variables, and the chi-squared test or Fisher's exact test for categorical variables. Differences between two time points within the same treatment arm were compared using paired t tests.

All tests were two-sided and a *P* value <0.05 was considered to indicate statistical significance. Data were analysed using STATA for Windows version 10.0 (StataCorp, College Station, Texas, USA).

Results

A total of 86 patients were randomly assigned to either the control group or the pretreatment group. Demographic characteristics did not differ significantly between both groups. As shown in **Table 1**, mean patient age, mean antral follicle count, and mean basal FSH and anti-Müllerian hormone concentrations were similar in both groups. The indications for fertility treatment did not differ between both groups (mostly male factor infertility and unexplained infertility). In both groups, the majority of patients were nulliparous (**Table 1**).

In the control group, three patients did not undergo oocyte retrieval, due to insufficient ovarian response resulting in monofollicular growth. In the pretreatment group, seven patients were excluded: protocol violation occurred in four patients, two patients developed a cyst and one further patient had insufficient ovarian response. In the control group, two patients did not reach the stage of embryo transfer because although embryos were available on day 3, there was no development to the blastocyst stage. In the pretreatment group, two patients did not have an embryo transfer, one because no embryo was available and the other for medical reasons (severe microcytic anaemia). The flow chart presenting these data is shown in **Figure 2**.

With regard to the outcome of ovarian stimulation in both groups, a longer duration of stimulation was observed in the pretreatment group (9.6 ± 1.4 days versus 8.6 ± 1.5 days in the control group; $P = 0.004$). The number of COC obtained at retrieval was similar in both groups. These data are summarized in **Table 2**.

Because of an increased risk of OHSS in four patients (two patients in the control group and two patients in the pretreatment group), final oocyte maturation was triggered

Table 1 Baseline characteristics of patients undergoing intracytoplasmic sperm injection.

	Control group (n = 42)	*Pretreatment group (n = 44)*
Age (years)	30.2 ± 3.0	29.2 ± 3.0
Weight (kg)	63.4 ± 9.4	63.7 ± 9.1
Height (cm)	167.6 ± 6.3	168.5 ± 7.3
BMI (kg/m^2)	22.3 ± 4.2	22.5 ± 3.0
AFC (*n*)	17.6 ± 9.4	18.7 ± 9.2
Basal FSH (IU/l)	6.9 ± 1.8	6.9 ± 1.9
AMH (μg/L)	4.4 ± 2.7	4.4 ± 2.1
Parity		
Zero	35/42	40/44
One or more	7/42	4/44
Indications		
Male factor infertility	24/42	26/44
Tubal factor infertility	3/42	3/44
Unexplained infertility	14/42	15/44
Endometriosis I and II	1/42	0/44

Values are *n*/total or means ± SD. There were no statistically significant differences as analysed by Student's t test for continuous variables and chi-squared test or a Fisher's exact test for categorical variables. AFC = antral follicle count; AMH = anti-Müllerian hormone; BMI = body mass index.

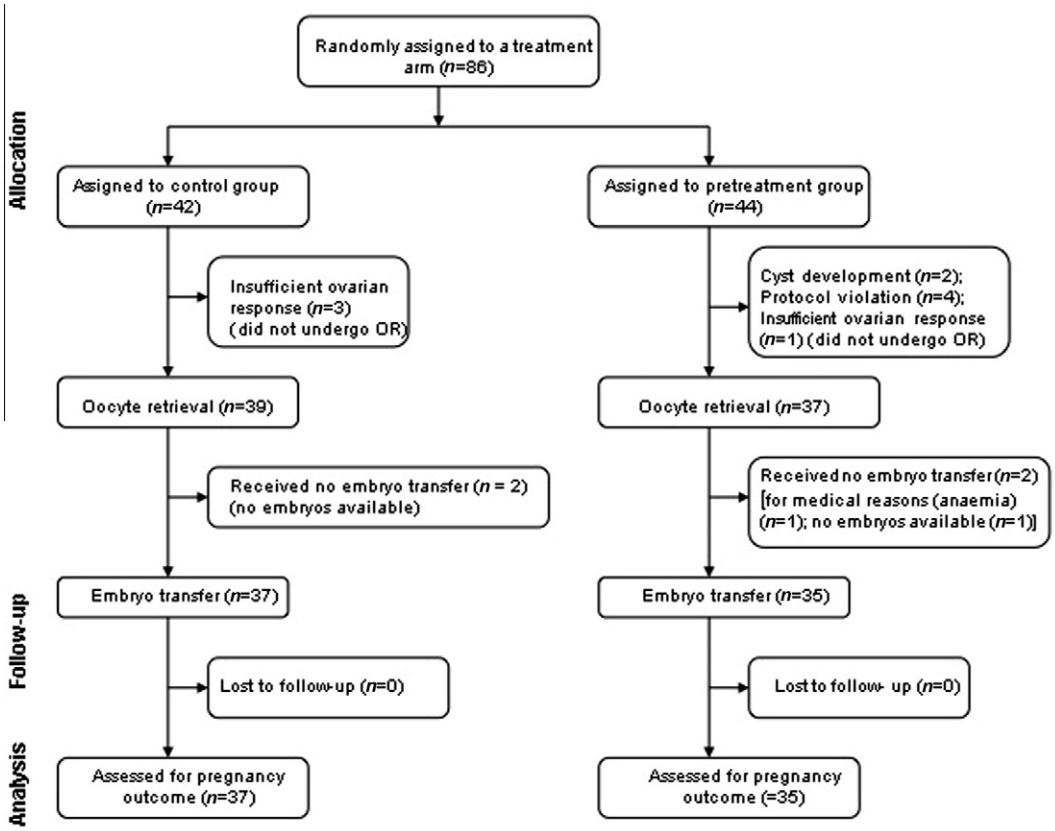

Figure 2 Study flow chart diagram. OR = oocyte retrival.

Table 2 Stimulation characteristics and embryological data.

	Control group (n = *39*)	*Pretreatment group* (n = *37*)	*Between-group difference*	P-*value*
Days of rFSH stimulation	8.6 ± 1.5	9.6 ± 1.4	1.0 (0.4 to 1.7)	0.004
Total rFSH (IU)	1295.0 ± 254.2	1485.1 ± 248.7	190.1 (75.1 to 305.1)	0.002
COC	12.2 ± 8.7	12.2 ± 6.2	0 (−3.5 to 3.5)	NS
MII oocytes	9.9 ± 7.8	10.0 ± 4.7	0.1 (−2.9 to 3.1)	NS
2PN oocytes	7.8 ± 6.5	8.4 ± 3.7	0.6 (−1.8 to 3.1)	NS

Values are means ± SD. Absolute between-group difference = pretreatment-group value − control-group value. *P*-value for Student's t-test for testing absolute difference between groups. COC = cumulus–oocyte–complexes; MII = metaphase II; 2PN = 2 pronuclei; NS = not statistically significant; rFSH = recombinant FSH.

with a single-dose of GnRH agonist (Decapeptyl) instead of HCG (Pregnyl) in these patients.

The proportion of patients undergoing an oocyte retrieval during the weekend was significantly lower in the pretreatment group (1/37) compared with the control group (8/39), with an absolute between-group difference of −17.8% (95% CI −31.5 to −4.1%; $P = 0.029$; **Table 3**). Only one oocyte retrieval occurred on a Saturday in the pretreatment group. The number of oocyte retrievals by day is shown graphically in **Figure 3**.

The clinical pregnancy rates per started cycle were similar in the pretreatment group (16/4, 38.1%) and the control group (17/4, 38.6%; between-group difference 0.5%; **Table 3**).

In the pretreatment group, pre-ovarian stimulation serum oestradiol concentrations were significantly higher compared with the control group (142.3 ± 67.1 pg/ml versus 43.7 ± 14.9 pg/ml in pretreatment and control group, respectively; between-group difference 98.6%; $P < 0.001$), whereas serum FSH concentrations were significantly lower in the pretreatment group (5.3 ± 2.1 IU/l versus 7.0 ± 2.0 IU/l in pretreatment and control group, respectively; $P < 0.001$). A summary of the hormone concentrations at the start of ovarian stimulation and on the day of HCG is shown in **Table 4**.

Table 3 Weekend oocyte retrievals and pregnancy rates.

	Control group	*Pretreatment group*	*Between-group difference (%)*
Patients undergoing oocyte retrieval during a weekend day (primary end point)	8/39 (20.5)	1/37 (2.7)	−17.8 (−31.5 to −4.1)[a]
Positive HCG			
Per started cycle	20/42 (47.6)	19/44 (43.2)	−4.4 (−25.5 to 16.6)
Per retrieval	20/39 (51.3)	19/37 (51.4)	0.1 (−22.4 to 22.6)
Per embryo transfer	20/37 (54.1)	19/35 (54.3)	0.2 (−22.8 to 23.3)
Outcome for patients with positive HCG test			
Biochemical pregnancy	2/20 (10.0)	1/19 (5.3)	−4.7 (−21.3 to 11.8)
Miscarriage	2/20 (10.0)	1/19 (5.3)	−4.7 (−21.3 to 11.8)
Clinical pregnancy	16/20 (80.0)	17/19 (89.5)	9.5 (12.8 to 31.8)
Clinical pregnancy rate			
Per started cycle	16/42 (38.1)	17/44 (38.6)	0.5 (−20.0 to 21.1)
Per retrieval	16/39 (41.0)	17/37 (45.9)	4.9 (−17.4 to 27.2)
Per embryo transfer	16/37 (43.2)	17/35 (48.6)	5.4 (−17.7 to 28.3)

Values are *n*/total (%). Absolute between-group difference = pretreatment-group value − control-group value. Fisher's exact or chi-squared test were used for testing absolute difference between groups. HCG = human chorionic gonadotrophin.
[a]$P = 0.029$.

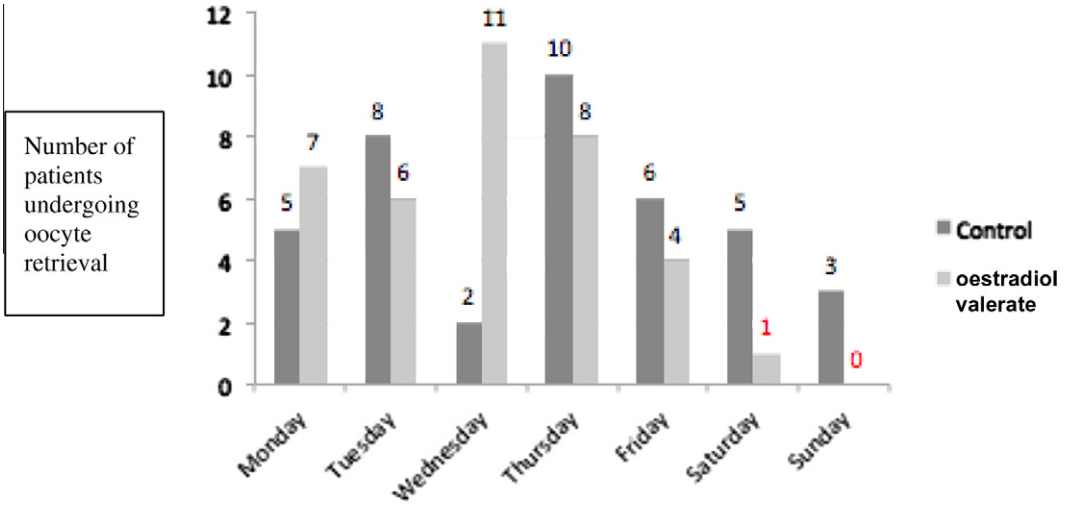

Figure 3 Oocyte retrieval by day in both treatment groups. The distribution of oocyte retrieval by day for patients randomized to control or pretreatment group was analysed by the Fisher's exact test ($P = 0.039$).

Discussion

Pretreatment with oestradiol valerate significantly reduces the proportion of oocyte retrievals during weekend days, without affecting the number of COC or clinical pregnancy rates. As far as is known, this is the first trial comparing the outcomes of GnRH-antagonist cycles with and without oestradiol valerate pretreatment in a randomized controlled fashion. Recently however, two randomized controlled trials were published comparing a GnRH-antagonist protocol with oestradiol pretreatment with a standard long agonist protocol (Guivarc'h-Levêque et al., 2010; Ye et al., 2009). GnRH-antagonist cycles with oestradiol pretreatment can facilitate good control of oocyte retrieval schedules with similar outcomes compared with long agonist cycles (Guivarc'h-Levêque et al., 2010). A disadvantage of that trial is the potential bias between GnRH agonists and antagonists, mainly in terms of the secondary endpoints, such as the number of oocytes and pregnancy rates. Moreover, there was no allocation concealment in this randomized study.

According to the protocol described here, scheduling oocyte retrievals has become more controllable, without prolongation of the follicular phase by delaying the administration of HCG, a practice that has been shown to result in lower pregnancy rates (Kolibianakis et al., 2004). In the present study, pregnancy rates were similar in the pretreatment group and the control group, although the study was not powered to detect differences in this outcome parameter. OCP pretreatment is increasingly being abandoned as a

Table 4 Serum hormone levels.

	At initiation of gonadotrophin stimulation				*On the day of hCG administration*			
	*Control group** (n = *42*)	*Pretreatment group** (n = *44*)	*Between-group difference (95% confidence limits) ***	*P-value*	*Control group** (n = *39*)	*Pretreatment group** (n = *37*)	*Between-group difference (95% confidence limits) ***	*P — value*
FSH, IU/L	7.0 ± 2.0	5.3 ± 2.1	−1.7 (−2.5 to −8.2)	<0.001	10.9 ± 2.4	11.2 ± 2.7	0.3 (−0.9 to 1.5)	0.610
P, ng/ml	0.8 ± 0.3	0.5 ± 0.2	−0.3 (−0.4 to −0.2)	<0.001	1.0 ± 0.3	0.9 ± 0.5	−0.1 (−0.3 to 0.1)	0.291
E2, pg/ml	43.7 ± 14.9	142.3 ± 67.1	98.6 (77.5 to 119.7)	<0.001	1482.7 ± 731.5	2168.9 ± 1450.7	686.2 (165.1 to 1207.3)	0.011
LH, IU/L	5.9 ± 1.9	6.2 ± 3.8	0.3 (−0.9 to 1.6)	0.647	2.4 ± 2.2	3.8 ± 4.5	1.4 (−0.2 to 3.0)	0.087

Values are means ± SD. Absolute between-group difference = pretreatment-group value − control-group value. HCG = human chorionic gonadotrophin; NS = not statistically significant.

planning tool, since this results in significantly lower pregnancy rates (Griesinger et al., 2010). It has been postulated that the gestagen component of the OCP could exert a negative impact on endometrial receptivity in the subsequent cycle. Alternatively, low endogenous LH concentrations 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). Whether oestradiol pretreatment has any adverse impact on the oocyte or the endometrium deserves further scrutiny in a larger study.

This study observed a higher total dose of gonadotrophins used for ovarian stimulation and a longer stimulation period, namely one additional day, in the oestradiol pretreatment group. These findings are consistent with previously published data, showing that the inhibitory effect of oestradiol on FSH in the phase of luteo-follicular transition may result in slower and more co-ordinated growth of the follicles before initiation of the stimulation (Fanchin et al., 2005; Hill et al., 2009).

Although this study was not powered to prove a significant difference in the number of COC, a similar number of COC were retrieved in both groups. Previous studies comparing GnRH antagonists with and without oestradiol pretreatment found a significantly higher number of COC when the pretreatment was administered (Cédrin-Durnerin et al., 2007; Fanchin et al., 2003; Smulders et al., 2010). At the start of the gonadotrophin stimulation, significantly higher oestradiol concentrations were observed in the pretreatment group, whereas serum hormone concentrations of progesterone and FSH were significantly lower, as a result of the negative feedback by the oestradiol administration. Conversely, significantly higher oestradiol concentrations were observed on the day of HCG administration; the other serum hormone concentrations were similar in both groups.

Triggering final oocyte maturation was performed with 10,000 IU HCG (Pregnyl) in the majority of the patients. Four patients (two patients in the control group and two patients in the pretreatment group), were administered a GnRH agonist (Decapeptyl) because of an increased risk of OHSS. These patients received a modified luteal-phase support scheme, i.e. a single dose of 1500 IU of HCG after oocyte retrieval, followed by vaginal progesterone as well as oestradiol valerate supplementation. This protocol, described by Humaidan (2009) and Humaidan et al. (2011), yields similar clinical pregnancy and delivery rates compared with a standard protocol with HCG trigger and standard luteal-phase support, and these four patients were therefore not excluded from the trial. None of the patients developed OHSS.

The clear practical advances of the protocol described here is associated with a lower financial cost than the GnRH-antagonist pretreatment protocol, which was previously described as a possible planning tool (Blockeel et al., 2011) even taking into account the increased gonadotrophin consumption of the oestradiol pretreatment protocol. Moreover, oestradiol valerate pretreatment has no teratogenic effects, which could be an important issue in case of an undiagnosed pregnancy. Finally, the potential side-effects of oestradiol valerate are mild. Hence, oestradiol pretreatment should be no threat to the patient-friendly label of the GnRH-antagonist protocol.

A possible disadvantage of avoiding oocyte retrievals during the weekend days is the increased workload caused by the embryo transfers during the weekend. Nevertheless, an oocyte retrieval involves more personnel, more time and more laboratory procedures (semen preparation, denudation of the COC and microinjection in case of ICSI) than an embryo transfer.

Although the sample size was met for the primary outcome, a larger sample size would have been of more interest, regarding the number of retrievals during weekend days, but also synchronization of the follicular cohort, number of retrieved oocytes and pregnancy outcome. However, the current study did not consider these secondary outcomes for the sample size calculations. Nevertheless, the data presented in the current paper will be helpful when planning future, adequately powered trials considering any of these endpoints as the primary outcome.

In conclusion, the ovarian stimulation protocol with GnRH antagonists lacks flexibility. Therefore, there is great

potential in developing tools to organize the workflow in an assisted reproduction clinic and to minimize the number of oocyte retrievals during weekend days. This trial has shown that scheduling GnRH-antagonist cycles using oestradiol valerate pretreatment is a potential planning tool: this modified GnRH-antagonist protocol leads to a reduction of oocyte retrievals during weekend days, without deleterious impact on the number of oocytes and the clinical pregnancy rates.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 10 August 2011; refereed 10 November 2011; accepted 15 November 2011.