Facts Views Vis Obgyn, 2015, 7 (4): 223-230

Original paper

Corifollitropin stimulation in combination with GnRH-antagonists after estradiol valerate pre-treatment. A pilot study on patient-friendly IVF

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Abstract

Objective: **To demonstrate the feasibility of scheduling an IVF cycle, without disadvantages, in the new patient friendly stimulation protocol using the long acting Corifollitropin Alfa, in combination with GnRH-antagonist protection and GnRH-agonist triggering.**
Study design: **Two groups of ten patients were admitted in the study. Both received the same stimulation protocol with Corifollitropin Alfa in combination with GnRH-antagonist protection. After ultrasound evaluation on day 7 individually dosed Menopur was added. For triggering final oocyte maturation GnRH-agonists were used. The only difference between the two groups was that in the study group, estradiol valerate 4 mg/day was given from day 25 of the preceding cycle for a period of 10 days, thus postponing the start of follicular growth.**
Results: **Scheduling the IVF stimulation by the administration of estradiol valerate 4 mg/day did not influence the hormonal curves, nor the embryological results in comparison to patients with the same stimulation, starting their stimulation at the beginning of menstruation. In this pilot study four out of ten patients turned out to be pregnant, demonstrating an acceptable pregnancy rate.**
Conclusion: **The combination of estradiol valerate 4 mg/day pre-treatment with the novel combination of Corifollitropin Alfa stimulation with GnRH-antagonist protection, individually topped off with Menopur, and triggered with GnRH-agonist proved to be a safe, patient-friendly (limited number of injections in comparison to classical IVF) (Patil, 2014) and efficient alternative to classical IVF stimulation protocols, allowing patients – and doctors – to schedule the treatment cycle to their convenience.**

Key words: **Scheduling, Corifollitropin Alfa, agonist-triggering, IVF, Progynova pre-treatment, patient-friendly IVF.**

Introduction

In vitro fertilisation (IVF) has always been a very stressful therapy for the patient. First of all the fertility problem itself induces a lot of distress for the couple. Moreover, the therapy with hormonal down-regulation, multiple injections for the stimulation, oocyte retrieval, and the possible side effects – such as overstimulation syndrome – form an additional burden for the patient and her partner. Over the last few years however, the emphasis of research moved a little from the technical aspects of the treatment towards, safer, easier and more patient-friendly protocols. This evolution occurred in several stages. A first step was the introduction of GnRH-antagonists in the ovarian stimulation protocols for assisted reproductive treatment to inhibit an endogenous LH-surge (Devroey et al., 2009a). These antagonists, which were generally started after five or six days of FSH-stimulation, cause immediate and reversible suppression of the production of gonadotropin, thus allowing a more physiological stimulation in the first phase of the controlled ovarian hyper stimulation for IVF. By

consequence a significantly lower probability of OHSS is achieved (Papanikolaou et al., 2006). Moreover, patients that underwent GnRH-antagonist protected stimulation, can be triggered for final oocyte maturation by the administration of GnRH-agonist instead of human chorionic gonadotropin (Humaidan et al., 2011). This method of induction of final oocyte maturation again reduced the risk of ovarian hyper stimulation syndrome dramatically, without negative impact on the final result in terms of life birth rate (Al-Inany et al., 2011).

A significant step towards a patient-friendly IVF-stimulation was the introduction of Corifollitropin Alfa (Devroey et al., 2009b), reducing dramatically the number of injections needed to achieve multifollicular growth. One single injection of Corifollitropin Alfa (Elonva®, MSD, Belgium) in the beginning of the cycle replaces six days of consecutive injections of FSH in the classical IVF-stimulation protocols. The dose of this single injection (100-150 microgram) is only dependent on body weight (Ledger et al., 2011), again simplifying the IVF-procedure (Decleer et al., 2014).

Still, one of the major burdens in fertility treatment was the planning of the therapy since the stimulation was depending on the hormonal cycle and the onset of menstruation of the patient. Already from the very early stages of the history of IVF, attempts were made to avoid being dependent on the random occurrence of spontaneous menses. This was important both for the patient, who wants to undergo reproductive treatment at her own convenience, and for the fertility team, that needs to organize the workload, including weekend-work. The introduction of GnRH-agonists, with the down regulation of the female hormonal cycle, was a first step in controlling the schedule of egg-retrieval (long protocol down regulation cycle). Another tool for scheduling the cycle being advocated was the use of oral contraceptive pills (Fluker et al., 2001) in the cycle preceding the actual treatment cycle. However, the administration of synthetic hormones obviously negatively influenced the pregnancy rate (Griesinger et al., 2008). Also, delaying the administration of hCG, thus influencing the time of oocyte retrieval, proved to be a hazardous procedure. Normally, triggering of final oocyte maturation is started, as soon as three follicles reach a diameter of 17 mm. Postponing this final oocyte maturation by two days resulted in a lower probability of ongoing pregnancy (Kolibianakis et al., 2004), probably due to changes in the endometrial circumstances (Kolibianakis et al., 2005).

Blockeel (2012) demonstrated the feasibility of scheduling the treatment cycle by the introduction of an estradiol valerate pre-treatment.

In this pilot study, a group of patients with estradiol valerate pre-treatment were stimulated with the combination of Corifollitropin Alfa, highly purified hMG, GnRH-antagonists, and finally GnRH-analogues as a trigger for ovulation. The hormone profiles, embryological data, and the clinical outcome were analysed and compared with the control group, which received exactly the same stimulation, without the estradiol valerate pre-treatment.

Materials and methods

Ten consecutive patients with a regular cycle of 28 days (+/- 1 day), between 24 and 36 years old and with tubal or male infertility were accepted in the study group after being informed on the study protocol, that has been revised by the institutional review board (O. L. Vrouw hospital, Aalst: Belgian registration B126201318796). Exclusion criteria for acceptance in the study group were age >38 years, BMI >35 kg/m^2 and major endocrinological pathology such as elevated prolactin, thyroid dysfunction and diabetes. From the andrological point of view TESE-patients were excluded to avoid fertilization problems, despite good oocyte quality. Patient characteristics such as age, cycle number and BMI were registered. Results were compared with another group of 10 patients, described in a pilot study (Decleer et al., 2014) (Table I) on the new stimulation protocol with Elonva® in

Table I. — Control group: group described in Decleer et al., Facts Views Vis Obgyn, 2014.

Baseline covariate	Study group Mean (SD)	Control group Mean (SD)
Age (years)	31.3 (3.22)	30.7 (3.70)
BMI (kg/m^2)	24.5 (2.99)	22.4 (3.94)
Duration stimulation (days)	13.1 (0.93)	13.4 (3.37)
Total FSH used (IU/L)	1042.5 (388.02)	1215.0 (1301.35)
IVF cycle	2.7 (2.11)	2.4 (1.17)

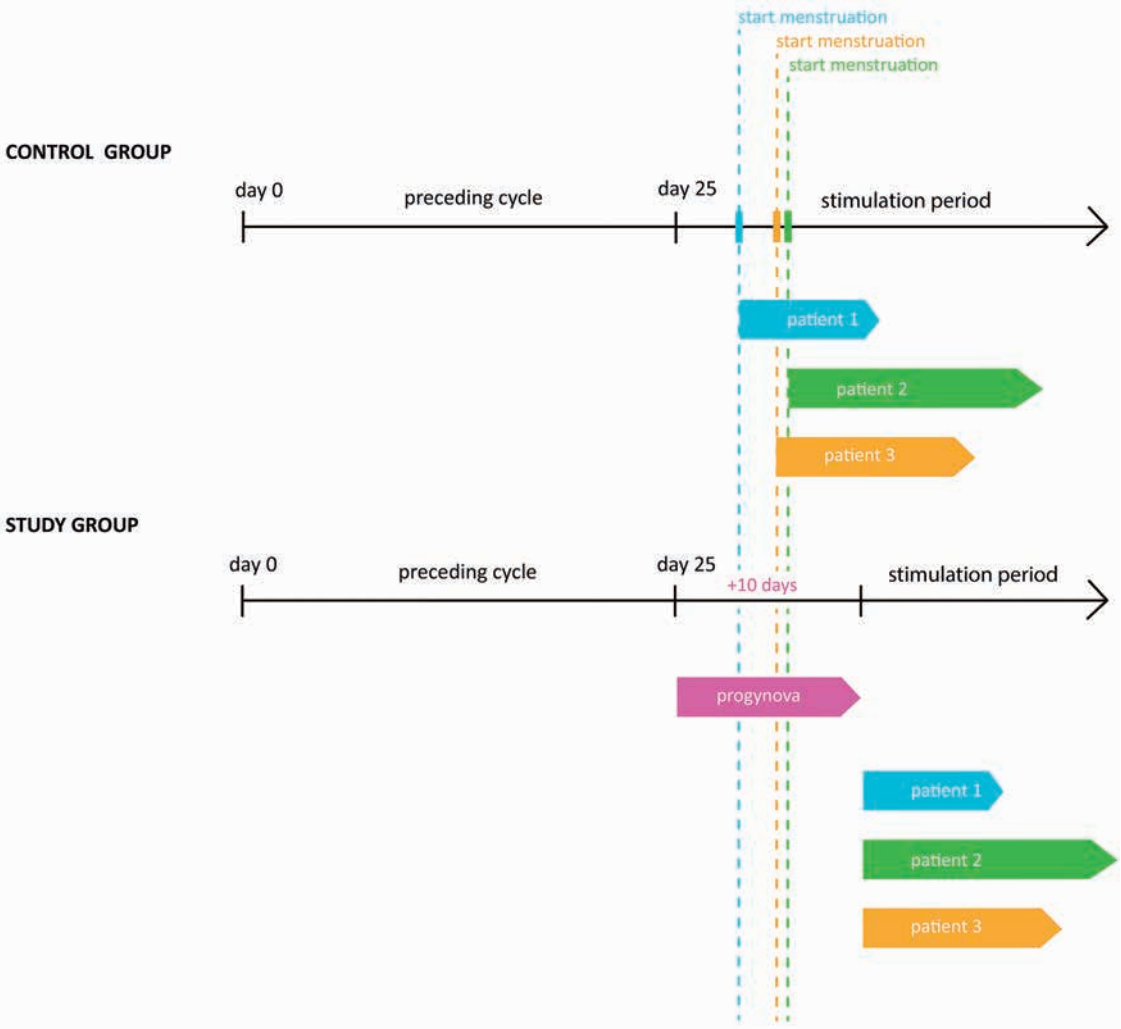

Fig. 1. — Ovarian stimulation for IVF: Study protocol

combination with highly purified hMG, GnRH antagonist protecting and GNRH analogues to trigger final oocyte maturation.

All patients included in the study group were given Progynova® 4 mg per day, from the 25th day of the proceeding cycle and for a period of 10 days. Regardless of the actual moment of the menstrual bleeding, ovarian stimulation was started only after finishing the 10 days period of Progynova® 4 mg per day intake (Fig. 1). The stimulation was started by the administration of 150 mg Elonva® single dose for patients with a body weight over 60 kg, and 100 mg of Elonva® for patients below that weight (Ledger et al., 2011). Six days later a GnRH-antagonist was administered by subcutaneous injection of ganirelix 0.25 mg/0.5 mL (Orgalutran®, MSD, Belgium) on a daily base. On day seven after the Elonva® injection, a first ultrasound evaluation measuring the size and number of follicles was performed. According to the individual reaction of the patient, an additional daily dose of highly purified menotropin (urinary extraction hMG, Menopur®, Ferring, Denmark) was added from day seven onwards, in combination with daily injections of GnRH-antagonist. The total dose of the medication as well as the duration (in days) of the stimulation were registered.

As soon as three follicles presented with a diameter of 17 mm or more, final oocyte maturation was induced by the injection of 0.2 mg of triptoreline acetate (Gonapeptyl®, Ferring, Denmark), in order to schedule oocyte retrieval 36 hours later (Humaidan et al., 2011; Kolibianakis et al., 2004). The support of the luteal phase was performed by injecting 1500 IU hCG immediately after oocyte retrieval (Fatemi et al., 2013). This injection was repeated seven days later as it is a standard in our centre for those patients who are not at risk for OHSS. Additionally, vaginal administration of 200 mg of micronized progesterone (Utrogestan®, Besins, France), three times a day, was started in the evening after oocyte retrieval.

The control group received exactly the same stimulation, starting on day two of the menstruation, without pre-treatment of estradiol valerate. Before the start of the stimulation by the administration of Elonva®, hormonal analysis was performed demonstrating basal values, especially progesterone being less than 1.5 μg/L (Hugues et al., 2010). Furthermore exactly the same stimulation protocol was followed in this control group as in the study group.

Embryo transfer took place three days after oocyte retrieval. One or two embryos were transferred, according to the Belgian law (one embryo in first IVF-attempt in patients less than 36 years old, one or two embryos for the second IVF-attempt depending on embryo quality, two embryos from the third attempt onwards) (Ombelet et al., 2005).

Hormonal analysis (estradiol, progesterone, LH and FSH) was performed before administration of Corifollitropin Alfa, at the time of triggering ovulation (administration of 0.2 mg of triptoreline), the day after triggering, the day of oocyte retrieval and from that day onwards every three days op to day 15 after oocyte pick-up.

Serum analysis of hCG and progesterone was performed 12 days after embryo transfer.

Furthermore embryological evaluation was performed by analysing and measuring the number of cumulus oocyte complexes (COC), the number of mature oocytes (Metaphase II oocytes), the number of fertilized oocytes (two-Pronuclei stage) on the day after oocyte retrieval, the number and quality of the embryos at the day of embryo transfer (= day three after oocyte retrieval), and the number of embryos that were good enough for cryopreservation (mature blastocyst stage with obvious inner cell mass and elaborated trophectoderm) at day 5 after fertilization.

Finally, the number of clinical pregnancies obtained was registered.

Descriptive statistics (i.e. mean and standard deviation) were calculated for the following continuous baseline parameters: age, BMI and IVF cycle; and for the observed outcomes: duration of stimulation, number of COC, number of MII oocytes, number of fertilized oocytes, number of transferred embryos, the number of cryopreserved embryos and the total amount of FSH. For exploratory purposes the mean levels for the baseline parameters and outcomes are compared between the control group and the study group by means of t-test and a non-parametric Wilcoxxon test. Hormone levels (mean +/- standard error) (i.e. estradiol, FSH, progesterone and LH) are displayed graphically in function of time for visual interpretation.

Results

The study population had an average age of 31.3 years (SD +/- 3.22) and a normal BMI of 24.5 kg/m² (SD +/- 2.99). The duration of the stimulation was 13.1 days (SD +/- 0.93) on average and a mean of 1042.5 IU (SD +/- 388.02) of highly purified hMG was given in addition to the Corifollitropin Alfa stimulation. All patients in the study group started on a pre-treatment with estradiol valerate, 4 mg per day, on day 25 of preceding cycle. Stimulation with Elonva® started on the day after the pre-treatment was finished, regardless of the time of the onset of menstruation. According to this procedure, the start of stimulation took place between day three and eight of the beginning of menstruation.

Hormonal analysis at the beginning of stimulation showed basal progesterone values for all patients (lower than 1.5 μg/L). Follicular growth proved to be adequate by the described stimulation, which was illustrated by relatively high maximum E2 values (mean 3485. 6 ng/L, SD +/- 586.03) (Fig. 2). When three or more follicles of 17 mm diameter were observed on ultrasound imaging final oocyte maturation was initiated by injecting 0.2 mg of triptoreline acetate.

No spontaneous LH-surge has been detected, demonstrating adequate suppression of pituitary gland. In one patient the moderate increase of the progesterone values at the end of the stimulation, and before starting final oocyte maturation, could be noticed (2.83 μg/L).

As also noticed in a previous study with Elonva® and GnRH-analogue triggering (Decleer et al., 2014), in the present study progesterone values stayed relatively low shortly after induction of final oocyte maturation (mean 6.1 μg/L, SD +/- 1.87), as well as on the day of oocyte retrieval. These values rose significantly after the administration of 1500 IU hCG (immediately after oocyte retrieval), up to a mean progesterone value of 87.4 μg/L (SD +/- 17.38) on day three after oocyte retrieval. All further serum progesterone values stayed high and well over 10 μg/L. The lowest value on day 12 after oocyte retrieval being 10.9 μg/L (mean 46.8 μg/L SD +/- 58.82) (Fig. 3).

Luteinizing Hormone (LH) levels returned to normal on the day of egg retrieval, after being very high approximately 12 hours following triggering ovulation by injecting 0.2 mg of triptoreline acetate (mean 78.2 IU/L, SD +/- 52.51) (Fig. 4). As shown in a previous study (Decleer et al., 2014), FSH profile shows a much lower peak, but therefore much slower return to basal values (Fig. 5).

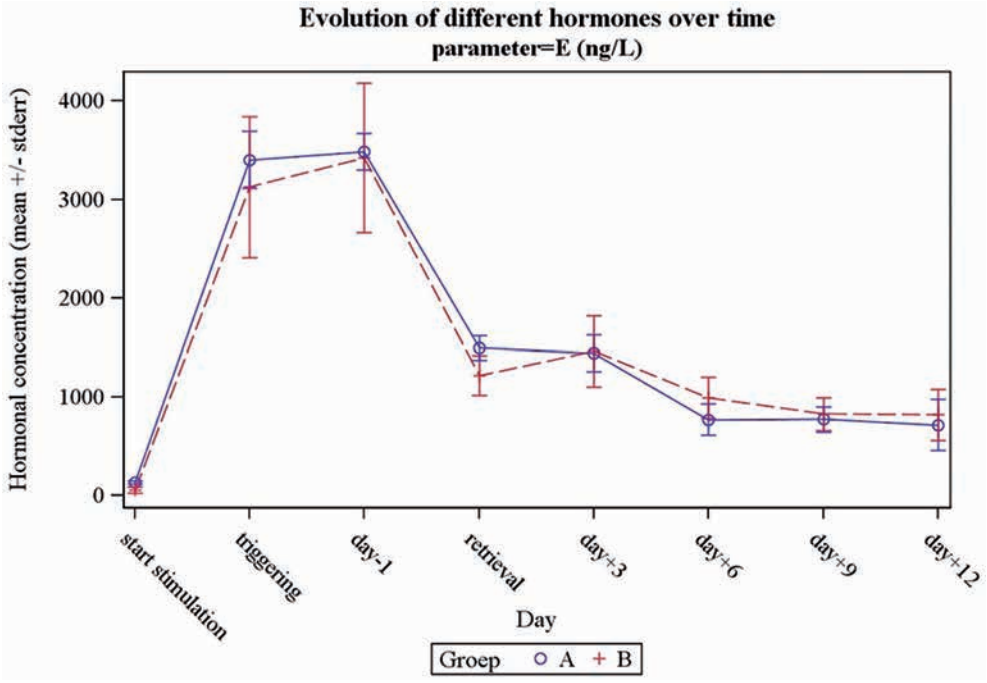

Fig. 2. — Evolution of different hormones over time: parameter = Estradiol (ng/L)

The number of COC's obtained at the moment of oocyte retrieval (mean 14.4, SD +/- 4.06) was adequate as was the number of mature oocytes (MII) that could be used for fertilization (mean 12.2, SD +/- 3.61). Fertilization rates were about 50%, with a mean number of 2PN fertilized eggs of 7.7 (SD +/- 3.65). In all patients a fresh embryo transfer took place with an average of 1.5 embryos being transferred.

In seven out of ten patients treated in this study, at least one embryo of excellent quality could be transferred. The definition of an excellent embryo in our study was an embryo of 6-8 cells on day three, a homogenous similar aspect of the blastomeres and less than 20% fragmentation. In four cases supernumerary good quality embryos could be cryopreserved in blastocyst stage.

Four out of ten patients in this observational study turned out to be pregnant. Ten days later all of them presented with a single intra-uterine gestational sac on ultrasound examination. There were no patients with clinical symptoms of OHSS.

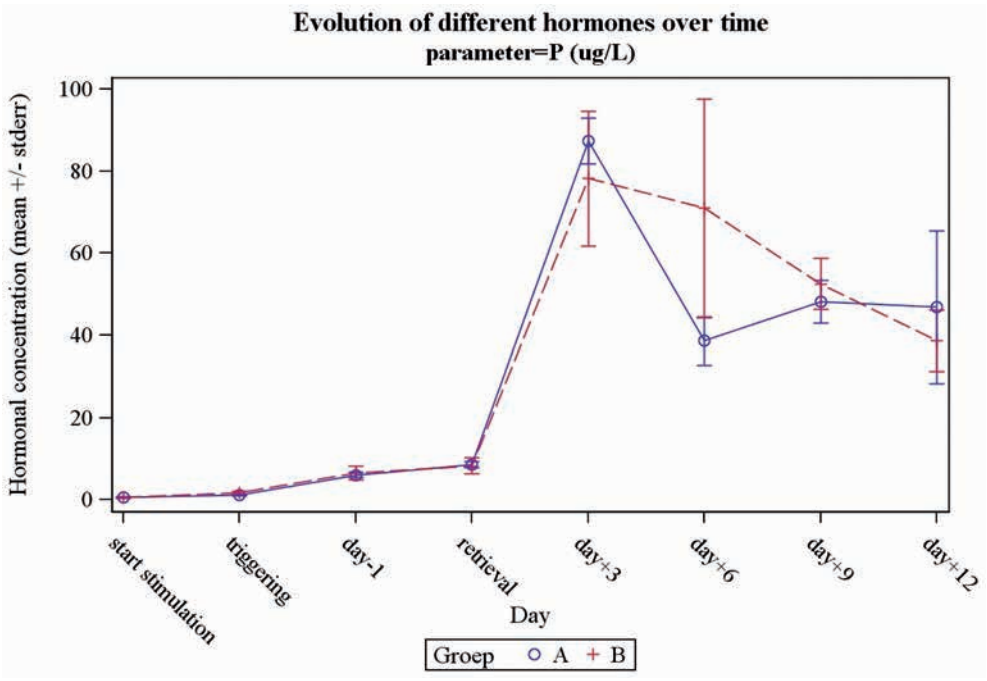

Fig. 3. — Evolution of different hormones over time: parameter = Progesterone (ug/L)

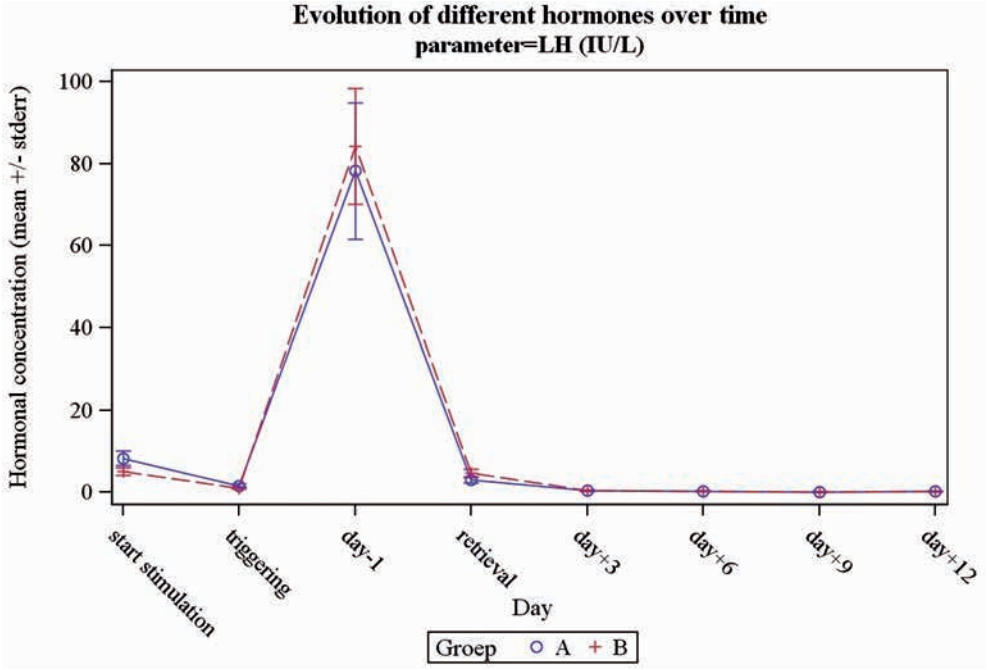

Fig. 4. — Evolution of different hormones over time: parameter = LH (IU/L)

The data of both the study group and control group are displayed in table II.

Discussion

The population analysis demonstrates completely normal parameters as far as age, body mass index and the rank of treatment cycle (mean 2.7, SD +/- 2.11) are concerned. There was no difference in the covariates between the group of patients with estradiol valerate pre-treatment and the control group. The stimulation period in both groups proved to show no statistical significant difference (13.1 vs 13.4 days respectively).

The hormone profiles in both groups showed adequate follicle growth with acceptable estradiol values proving adequate follicle maturity. The average maximum estradiol value per obtained COC was 242.1 ng/L for the estradiol valerate pre-treatment group, as it was 297. 6 ng/L for the control group. As shown before (Decleer et al., 2014) in this kind of stimulation the estradiol levels returned

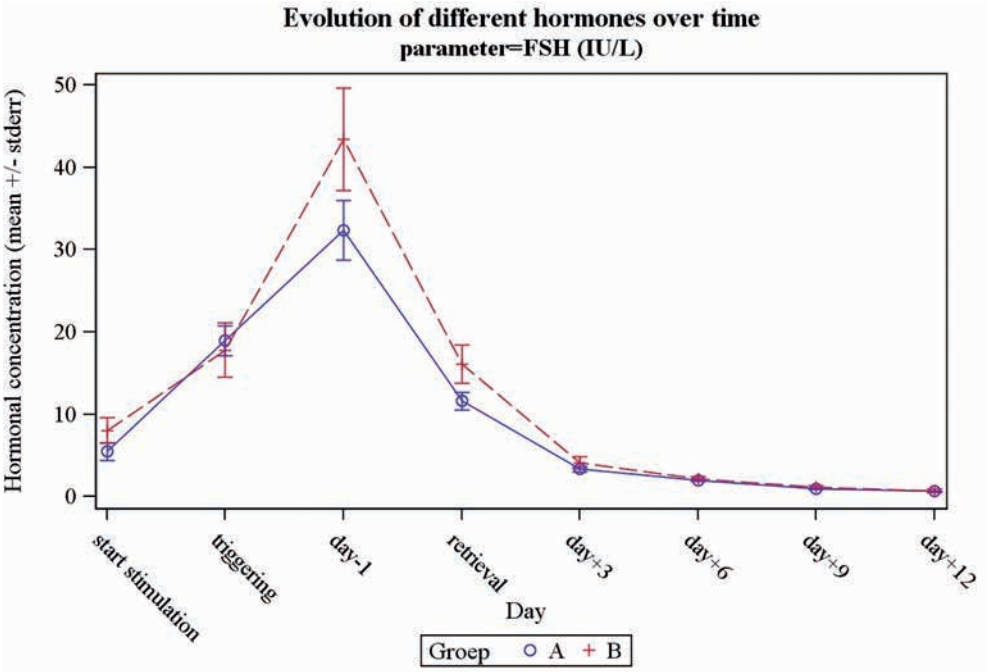

Fig. 5. — Evolution of different hormones over time: parameter = FSH (IU/L)

Table II. — Control group: group described in Decleer et al., Facts Views Vis Obgyn, 2014.

Endpoint	Study group Mean (SD)	Control group Mean (SD)
#COC*	14.4 (4.06)	11.5 (5.42)
#MII*	12.2 (3.61)	9.7 (5.01)
#2PN*	7.7 (3.65)	5.4 (3.37)
#ET*	1.5 (0.53)	1.8 (0.63)
#cryo*	1.4 (2.07)	0.9 (1.73)
Embryo quality A**	7 (70%)	5 (50%)
CPR**	4 (40%)	4 (40%)

*Mean (SD = standard deviation).
**Number (percentage).
COC: cumulus oocyte complexes; MII: metaphase II oocytes; 2PN: normal fertilization with 2 pronuclei; ET: embryo transfer; CPR: Clinical Pregnancy Rate.

rapidly to normal physiological levels in the implantation period, in comparison to cycles triggered by the administration of hCG.

Progesterone levels remained high in all cases up to day nine after pick-up. However, in the estradiol valerate pre-treatment group, on day 12 after oocyte retrieval, a decrease of the progesterone levels under or equal to 20.0 μg/L occurred in three out of ten patients. As has been demonstrated (Decleer et al., 2014) the rise of progesterone after ovulation induction GnRH-triggering started relatively slowly and only came to full expression only after Pregnyl® 1500 IU was added, i.e. immediately after oocyte retrieval. The low progesterone values at the beginning of the implantation phase in the estradiol valerate pre-treatment group might be illustrating an inadequate support of the luteal phase of the cycle, if GnRH-analogues alone are used for final oocyte maturation. Also in the control group in two cases the progesterone below the level of 20 μg/L was noticed. Obviously this decrease of progesterone levels has nothing to do with the estradiol valerate pre-treatment, but al the more with the support of luteal phase in GnRH-analogue triggering. A more active support of the luteal phase by a higher dose of hCG both on the day of oocyte retrieval and seven days later, added to the vaginal administration of micronized progesterone 3 times 200 mg daily (Geber et al., 2007), might be considered. It is obvious that neither in the group with estradiol valerate pre-treatment nor in the control group, pregnancy occurred in those patients that had an early decrease of progesterone levels in the implantation phase.

The analysis of the embryological features turns out to be very interesting. The cumulus oocyte complexes turned out to be slightly higher in the estradiol valerate pre-treatment group than in the control group. Also the number of mature oocytes (MII oocytes) seemed to be slightly higher. Furthermore the number of fertilized eggs, as well as the number of good quality embryos and the number of frozen embryos, was slightly higher in the estradiol valerate pre-treatment group than in the control group. However, none of these differences were statistically significantly different. Further investigation is needed to investigate whether this tendency to better embryological parameters, probably due to synchronisation of the follicles, is turning out to be significant.

According to Belgian law, the number of transferred embryos was limited according to age and cycle number, and this was similar in the study and control group. The pregnancy rates in both groups were four out of ten patients.

Conclusion

The implementation of a pre-treatment of estradiol valerate 4 mg per day over a ten day period, starting from day 25 of the preceding cycle, thus postponing the start of a new hormonal cycle, does not have any implication on the hormonal profiles and the embryological data of a patient population stimulated with Corifollitropin Alfa followed by individually dosed highly purified hMG, in combination with GnRH-antagonist protection and induction of final oocyte maturation by GnRH-agonist, combined with low dosed hCG support of the implantation phase. Embryological data and pregnancy rates proved to be the same in both groups. More scientific research is needed on the control of progesterone production in the luteal phase, concerning the support of the implantation

chances of the embryos. This estradiol valerate pre-treatment offers new possibilities in scheduling IVF patients.

Acknowledgements

A special contribution has been made by Mrs. Lieve Declercq and Mrs. Caroline Van de steene, who coordinated the patients' follow up, and the collection of all hormonal and embryological data presented in this study, as well as the lay-out of the paper and to Mr. Tim Decleer for the editing.

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