A new approach for ovarian stimulation in IVF using Corifollitropin Alfa in combination with GnRH analogues to trigger final oocyte maturation. A pilot study.

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Abstract

A pilot study of 10 patients undergoing IVF stimulation, using the new combination of Corifollitropin Alfa with highly purified hMG and GnRH antagonists has been performed, whereas final oocyte maturation was induced by GnRH analogues. The hormonal profiles were analyzed, as well as the clinical outcome. All patients were recruited between March 1st 2013 and June 30th 2013. They were all younger than 38 years, had a normal BMI (between 18,0 and 32,0) and did not have more than three previous IVF stimulations. The combination of long acting FSH with hphMG, and under protection of GnRH antagonists against spontaneous LH-surge, provided a normal hormonal profile for estradiol, progesterone, LH, and FSH. The average oocyte quality and embryo quality were excellent, which resulted in four pregnancies out of ten. We conclude that the described combination is a safe, efficient, and patient friendly alternative for the classical IVF stimulation.

Key words: Corifollitropin Alfa, GnRH analogue triggering, hCG support luteal phase, ovarian stimulation, IVF.

Introduction

The procedure of In Vitro Fertilization (IVF) mostly starts with ovarian stimulation in order to induce multiple follicle maturation (Morrell et al., 1971; Lunenfeld, 2004). In the early days of IVF, FSH extracted from the urine of menopausal women was used for this purpose (Donini and Montezemolo, 1949; Howles et al., 1992). Nowadays recombinant FSH administration is the standard in our centre for "controlled" ovarian stimulation (Hayden et al., 1999).

Parallel to the stimulation of the ovaries, a spontaneous LH-surge by the pituitary of the patient should be prevented. This was originally obtained by the administration of GnRH agonists (Lambalk et al., 2006), either in the form of a nasal spray (Ho et al., 2008), a daily injection or a long-acting product (Gonen et al., 1991). This also offered the possibility of down regulating the hormonal cycle

(Daya, 2000), and even scheduling IVF stimulations and embryology work (Rombauts et al., 2006).

However, the disadvantages of these therapies with down regulation were the long lasting stimulations and the numerous injections. The invention of a long-acting FSH analogue, Corifollitropin Alfa (Elonva®, MSD, Belgium) changed the burden of ovarian stimulation substantially, since the first week of daily FSH-injections was replaced by one single subcutaneous injection (Devroey et al., 2009a). According to the individual reaction of the patient on this single injection of Corifollitropin Alfa, additional FSH can be given in order to promote adequate follicle growth. On the other hand, the introduction of GnRH antagonists, with almost immediate blockage of the endogenous production of LH added another opportunity to simplify stimulation, and to reduce the physical burden for the fertility patient (Albano et al., 1998; Olivennes et al., 1994; The European

Orgalutran Study Group et al., 2000; Devroey et al., 2009b). Indeed, this medication only has to be started, once the follicle growth reached a certain level, e.g. day six of the stimulation (Hamdine et al., 2013) or at the last if the leading follicle reaches 16 mm (Tannus et al., 2013). Another advantage of this kind of blockage of the endogenous LH production, GnRH agonists could be used to induce the final maturation of oocytes (Kol and Humaidan, 2010), a procedure which was generally realized by the administration of hCG (Pregnyl®, MSD) (Melo et al., 2009). This combination of "down regulation" of the endogenous hormones from the pituitary gland by GnRH antagonists and the induction of final maturation of the oocytes by GnRH agonists possibly improved oocyte quality (Humaidan et al., 2009). Also the risk for ovarian hyperstimulation syndrome, the most common complication of IVF therapy, is reduced (Melo et al., 2009; Devroey et al., 2011).

In this pilot study, the unique combination of Corifollitropin Alfa, highly purified HMG, GnRH antagonist and finally GnRH analogues as a trigger for ovulation was examined.

Materials and Methods

Ten consecutive patients, between 24 and 36 years of age with tubal or male infertility were accepted in the study group after being informed on the study protocol, which has been revised by the institutional review board. Exclusion criteria were age > 38 years, BMI > 35 kg/m² and major endocrinological pathology such as elevated prolactin, thyroid dysfunction and diabetes. From the andrological point of view TESE patients were excluded (Devroey et al., 1995). Patient characteristics such as age, cycle number and BMI were registered.

The stimulation was started on day two of the menstruation, after hormonal analysis demonstrating basal values, especially progesterone being < 1.5 μg/L (Hugues et al., 2010). Corifollitropin Alfa was administered subcutaneously, according to the body weight of the patient. 100 mg was given in patients weighing less than 60 kg, whereas those over 60 kg bodyweight received 150 mg (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 basis. On day seven after the Elonva injection, a first ultrasound evaluation measuring the size and the number of follicles was performed. According to the individual reaction of the patient, 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 (Ganirelix®, Serono, Belgium) (Coomarasamy et al., 2008). The total doses of medication, as well as the duration of the stimulation (in days) were registered.

As soon as three follicles measured 17 mm in diameter were present, 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 (Kolibianakis et al., 2004; Morley et al., 2012). 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 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.

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 < 36 years old, one or two embryos for the second IVF attempt, depending on embryo-quality, two embryos from the third attempt onwards (Salame et al., 2007)).

Hormonal analysis (estradiol, progesterone, Luteinizing Hormone and Follicle Stimulating Hormone) was performed before administration of the 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 up to day 15 after oocyte pick-up.

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

Also an 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 was good enough for cryopreservation.

Finally, the number of clinical pregnancies obtained was registered.

Statistical analysis was performed on these data and the hormonal values were plotted in graphics for easier analysis of the hormonal evolution.

Results

The study population had an average age of 33.96 years (SD +/- 3.70) and a normal BMI of

22.41 kg/m² (SD+/-3.94). The duration of the stimulation period was 13.40 days (SD+/-3.73) and a mean 1215 IU (SD+/-1301.35) of hphMG had to be added to the Corifollitropin Alfa stimulation.

All patients started with basal hormonal values, all basal progesterone values were < 1.5 µg/L. The follicular growth proved to be adequate by the described stimulation which was illustrated by relatively high maximal E2-values (mean 3124.95 ng/L, SD +/-2268.40) at the time three or more follicles of 17 mm diameter were observed on ultrasound imaging, and final oocyte maturation was initiated by injecting 0.2 mg of triptoreline-acetate. No spontaneous LH-surge has been detected, demonstrating adequate suppression of the pituitary gland. However, in two patients moderate increase of the progesterone values, at the end of the stimulation, and before starting final oocyte maturation could be noticed. In one of these two patients this seemed to have had a negative effect on embryo quality, the second one however had normal fertilization and embryo quality and ended up with an on-going pregnancy.

Progesterone values stayed relatively low shortly after induction of final oocyte maturation (mean 6.57 µg/L, SD+/-5.50), as well as on the day of oocyte retrieval (mean 8.28 µg/L, SD+/- 6.12) and only rose significantly after the administration of 1500 IU hCG. (Progesterone measurement on day three after oocyte retrieval (mean78.21 µg/L, SD +/- 52.05)). All further serum progesterone values stayed high for the full implantation period, the lowest value on day 12 after oocyte retrieval being 14.2 µg/L (mean 38.76 µg/L, SD+/-22.31).

Luteinizing Hormone (LH) levels returned to normal very soon after egg-retrieval, after being very high approximately 12 hours following triggering ovulation by injecting 0.2 mg of triptoreline-acetate (mean 84.20 IU/L, SD +/-44.51). On the contrary, the Follicle Stimulating Hormone (FSH) profile shows a much lower peak, but therefore a much slower return to basal values (Fig. 1, 2, 3, 4).

The number of COC's obtained at the moment of oocyte retrieval (mean 11.50, SD+/-5.42) was adequate, as was the number of mature oocytes (MII), usable for fertilization (mean 9.70, SD+/-5.01). Fertilization rates (55%) however, were a little disappointing with a mean number of 2PN embryos of 5.40 (SD +/- 3.37). In all patients, a fresh embryo transfer took place with an average of 1.8 embryos being transferred (SD+/- 0.63). In eight out of ten patients treated in this study, at least one embryo of excellent quality was transferred. These embryos contained 6-9 cells on day three. They showed a homogenous, similar development of the blastomeres and less than 20% fragmentation.

As a consequence of the relatively low number of embryos, only in three cases supernumerary good quality embryos could be cryopreserved.

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. Only one patient presented with moderate complaints of ovarian hyperstimulation syndrome. These complaints disappeared spontaneously after a few days.

Discussion

The population analysis demonstrates completely normal parameters as far as age, body mass index and the rank of treatment cycle (mean 2.55, SD +/-1.21) are concerned. The stimulation period with this new combination of hormonal stimulating agents has proven to mimic natural cycle evolution with a stimulation period of 13.4 days.

The hormonal profiles show an adequate follicle growth with acceptable estradiol values that never reached excessive levels. The average max E2 value per obtained COC was 297 ng/L, proving adequate follicle maturity. Interestingly however, is the fact that estradiol levels returned quite rapidly to normal physiological ones in the implantation period as compared to cycles triggered by the administration of hCG, indicating may be a still insufficient support of the second phase of the cycle.

Progesterone levels on the other hand remained high up to the end of the luteal phase, thus demonstrating adequate corpus lutein function, and sufficient support of the secretory endometrium. However, the rise of progesterone after ovulation induction started relatively slow, and only came to full expression after Pregnyl® 1500 IU was added, i.e. immediately after oocyte retrieval. This might be illustrating an inadequate support of the luteal phase of the cycle if GnRH analogues alone are used for final oocyte maturation. However, at the time of embryo transfer, the levels of progesterone already reached adequate ones, and remained high for the remaining part of the cycle. This indicates adequate support for the entire luteal phase by the combined injection of Pregnyl® 1500 IU both on the day of oocyte retrieval and 7 days later, added to the vaginal administration of micronized progesterone three times 200 mg daily (Geber et al., 2007).

In two cases, a moderate increase of pre-ovulatory progesterone was seen, although without the smallest increase of LH. One of these two cases later happened to have the worst embryo quality, may be indicating a devastating effect of luteinized
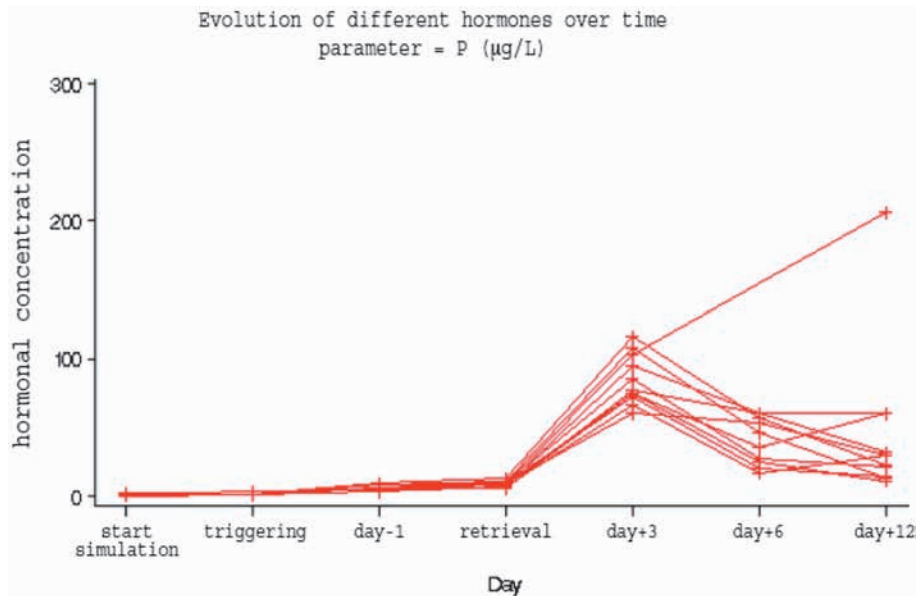
Progesterone levels raised significantly after administration of hCG.

***Fig. 1.*** — Progesterone values over time.
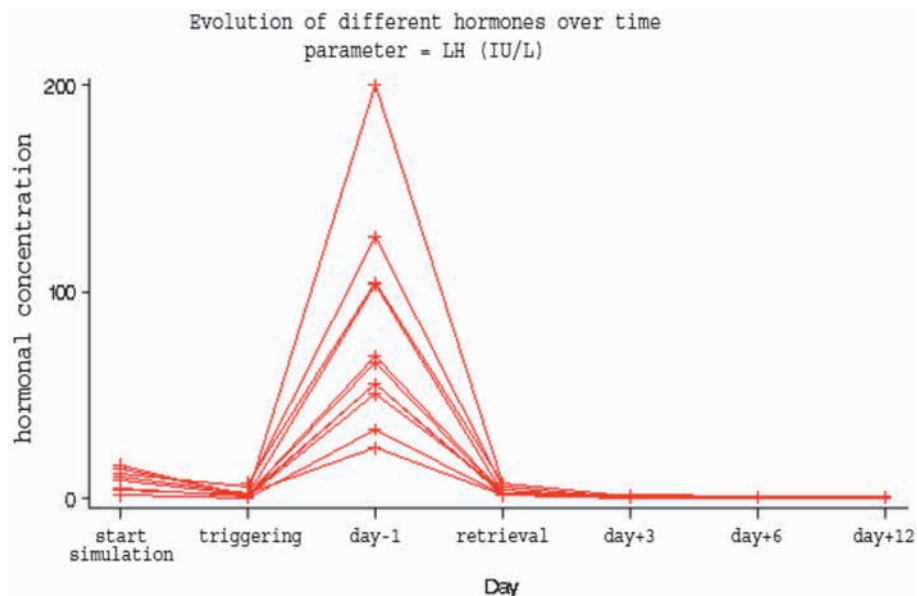
LH peaks high 12 hours after triggering ovulation and normalizes soon after oocyte retrieval.

Fig. 2. — LH values over time.

eggs (Bosch et al., 2010; Ochsenkühn et al., 2012; Venetis et al., 2013). However, the second case, on the contrary, showed very good embryo quality and eventually, at the end of the cycle, turned out to be pregnant.
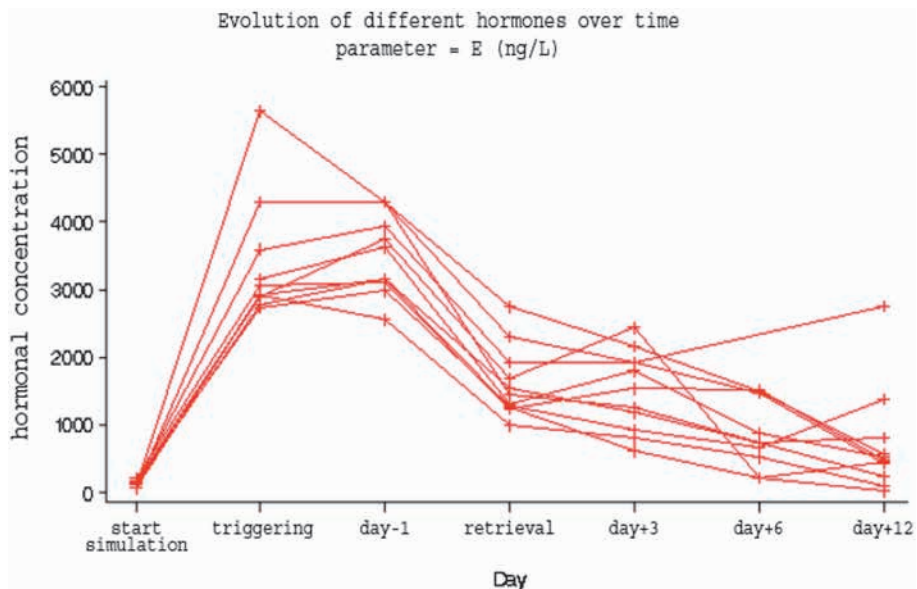
The LH-surge, induced by the triptoreline-acetate administration was clear-cut and very explicit, accompanied by a somewhat less expressed and therefore a little longer lasting FSH rise. Both values returned to normal shortly after oocyte retrieval.

The number of obtained oocytes was very adequate. Also the maturity of the gametes was more than sufficient with a percentage of 84% metaphase II oocytes. A little disappointing in this small population was the percentage of fertilized eggs. Indeed, only 55% (5.4/9.7) of all mature oocytes were fertilized, or 47% of all oocytes recovered at the time of oocyte retrieval (5.4/11.5). Whether this fertilization rate was caused by the stimulation or by the method of induction of final

Evolution of different hormones over time
parameter = LH (IU/L)
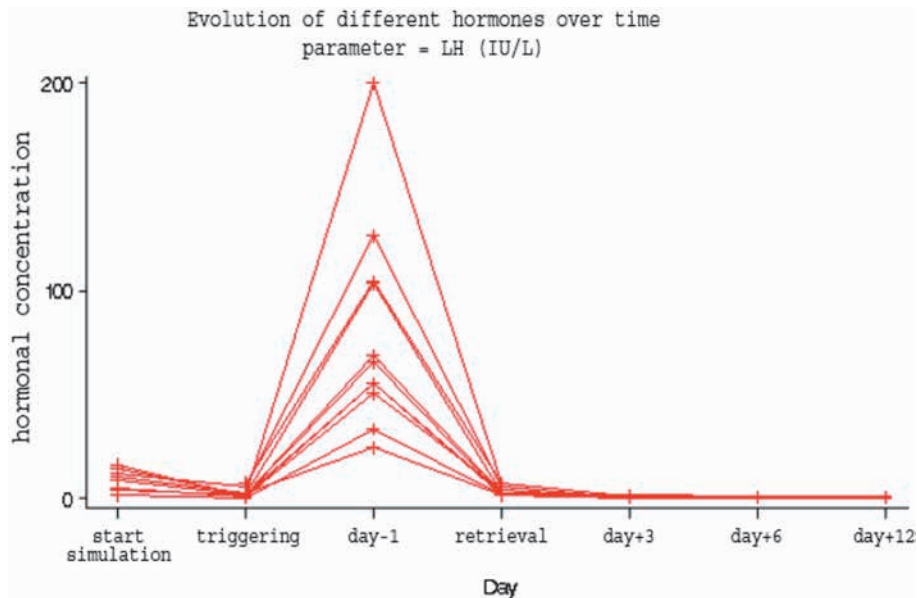
FSH peaks low returns slowly to basal value.

Fig. 3. — FSH values over time.

Evolution of different hormones over time
parameter = E (ng/L)

A relatively high max E2 value at time of triggering and quick return to basal level.

Fig. 4. — Estradiol values over time.

oocyte maturation needs further investigation. On the other hand the quality of these fertilized eggs was higher than average, which leads to nine out of ten patients having at least one embryo of superior quality transferred in the six to nine cell-stage, with homologous blastomere division, and only minor fragmentation levels. This resulted in an acceptable clinical pregnancy rate of 40% (4/10). No ectopic pregnancies were seen in this limited study population.

No serious ovarian hyper stimulation was observed in this population although one patient presented with minor overstimulation symptoms disappearing spontaneously after a few days.

Conclusion

The administration of Corifollitropin Alfa with subsequent individually dosed highly purified HMG stimulation, in combination with GnRH antagonist

protection, and induction of final oocyte maturation by GnRH agonists, combined with low dose hCG support of the implantation phase proved to be a safe and efficient alternative to classical ovarian stimulation for IVF. However, further scientific work is needed on the control of progesterone production both during the follicular growth as in the early secretory phase.

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