

# **Follicular and Luteal Phase Aspects of Ovarian Stimulation for In Vitro Fertilization**

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Thesis, Erasmus University, Rotterdam, the Netherlands

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# **Follicular and Luteal Phase Aspects of Ovarian Stimulation for In Vitro Fertilization**

**Folliculaire en Luteale Fase Aspecten van Ovariële Stimulatie voor In Vitro Fertilisatie**

## **PROEFSCHRIFT**

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**List of abbreviations**

$\alpha$	alpha
ANOVA	analysis of variance
AUC	area under the curve
$\beta$	beta
BMI	body mass index
$C_{\max}$	maximum concentration
CTP	carboxy-terminal part
CV	coefficient of variation
D	day(s)
DNA	deoxyribonucleic acid
$E_2$	estradiol
EIA	enzyme immunoassay
EMC	Erasmus MC Rotterdam
ESHRE	European Society of Human Reproduction and Embryology
ET	embryo transfer
FSH	follicle stimulating hormone
GnRH	gonadotrophin releasing hormone
GnRHa	gonadotrophin releasing hormone agonist
GnSAF	gonadotrophin surge attenuating factor
H	human
H	hour(s)
HCG	human chorionic gonadotrophin
HMG	human menopausal gonadotrophin
ICSI	intra cytoplasmatic sperm injection
i.e.	Id est (it is)
im	intramuscular
IU	International unit
IUI	intra uterine insemination
IVF	in vitro fertilization
$Kg/m^2$	kilograms per square meter
L	liter
LH	luteinizing hormone
M II	metaphase II
mg	milligram
ml	milliliter
mm	millimeter
nmol	nanomol

## List of abbreviations

No	number
OHSS	ovarian hyperstimulation syndrome
OPU	oocyte pick up
P	progesterone
pg	picogram
pmol	picomol
r-	recombinant
rec	recombinant
rFSH	recombinant FSH
RIA	radio immuno assay
sc	subcutaneous
SD	standard deviation
SE	estimated with a precision
TVS	trans vaginal ultrasound
µg	microgram
VUB	Academisch Ziekenhuis Vrije Universiteit Brussel
wk	week(s)
yrs	year(s)



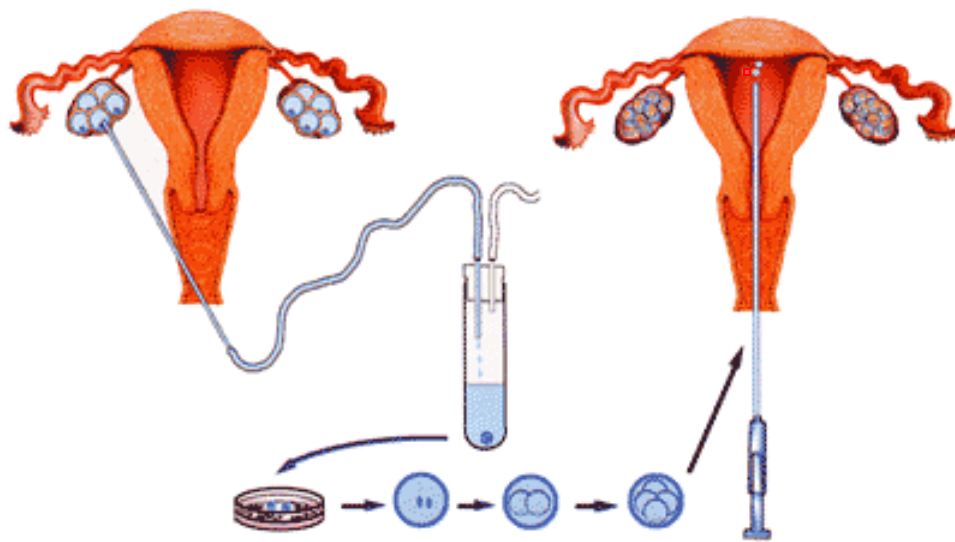


## **Chapter 1:**

### **Introduction and objectives.**

## General introduction

In 1976, the first pregnancy after in vitro fertilization (IVF) (see Fig. 1) was established. Unfortunately this pregnancy turned out to be extra-uterine, but on July 25th 1978, the first IVF baby, a healthy girl named Louise Brown, was born (Steptoe and Edwards 1978 [▼](#)). In the first years of IVF, the technique was restricted to the treatment of women with tubal infertility. Later on, the indications for IVF expanded to include male infertility, endometriosis and subfertility of unknown cause, 'idiopathic' subfertility. Since the early 1990's, even couples with severe male infertility can be treated with IVF combined with intra cytoplasmic sperm injection (ICSI) (Van Steirteghem et al., 1988 [▼](#)). World wide, 500.000 IVF and ICSI cycles are performed each year, resulting in the birth of 20.000 babies (ASRM 2002 [▼](#); Nyboe Andersen et al., 2004 [▼](#)). It is estimated that currently, around 2.5 million babies have been born world wide following IVF. In the Netherlands, 2% of all babies born are conceived by IVF (Kremer et al., 2002 [▼](#)).



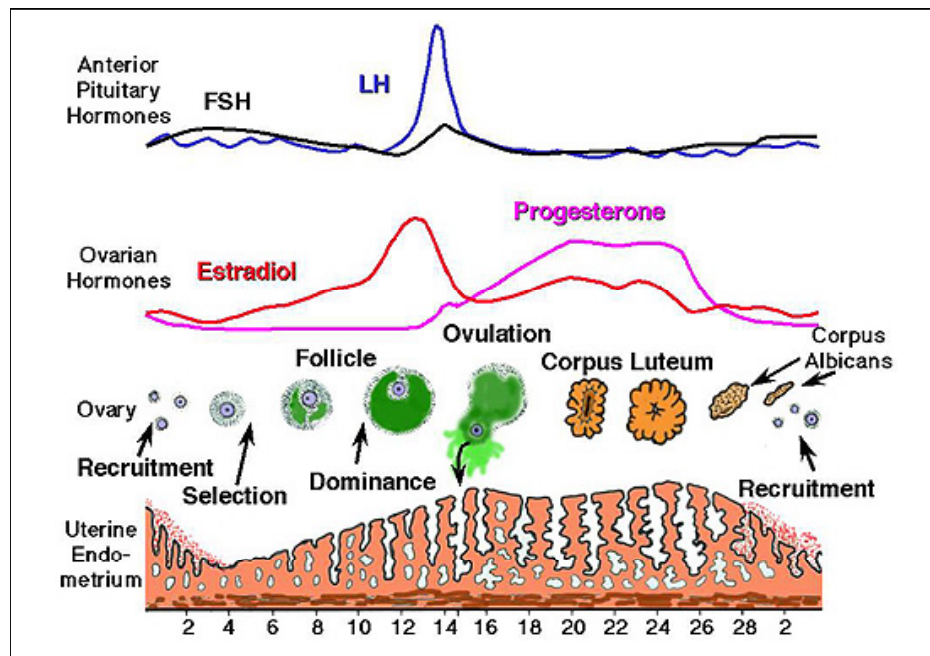
**Figure 1.** Schematic representation of the procedure of in vitro fertilization (from: [klc.ne.jp/page/ivf\\_et.html](http://klc.ne.jp/page/ivf_et.html))

In the last 30 years, IVF treatment itself has evolved in many ways. The first successful IVF attempt was performed in the natural cycle, while today women are routinely treated with ovarian hyperstimulation and luteal phase support. Improved ovarian hyperstimulation regimens have resulted both in increased pregnancy chances and in improved patient convenience and safety.

## The normal menstrual cycle

### *The follicular phase*

Gonadotrophin releasing hormone (GnRH) is released from the hypothalamus in a pulsatile manner and stimulates the synthesis and the release of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) by the anterior pituitary gland. In the early follicular phase FSH levels rise. The initial growth of primordial follicles in the ovaries is thought to be independent of FSH and commences in a random fashion (Peters et al., 1975 [▼](#)). The majority of these primordial follicles go into atresia, only those antral follicles that happen to be at a more advanced stage of maturation during the inter-cycle rise in FSH (levels surpassing the so called threshold for ovarian stimulation) gain gonadotrophin dependence and continue to grow (Fauser and van Heusden 1997 [▼](#); McGee and Hsueh



**Figure 2.** The normal menstrual cycle (from: [www.wisc.edu/ansci\\_repro/lec/lec\\_11/lec11fig.html](http://www.wisc.edu/ansci_repro/lec/lec_11/lec11fig.html))

2000 [▼](#)). During the mid and late follicular phase, FSH serum levels steadily decrease below the threshold due to negative estradiol ( $E_2$ ) feedback at the hypothalamic-pituitary axis (Baird 1987 [▼](#)). Moreover, a rise in inhibin B serum levels takes place early in the

follicular phase, suggesting that it is secreted by a recently recruited cohort of follicles in response to FSH (Laven and Fauser 2004[▼](#)). This rapid rise in inhibin B occurs immediately after the inter-cycle rise in FSH (Groome et al., 1996[▼](#)). It may be proposed that inhibin B limits the duration of the FSH rise through negative feedback at the pituitary level (Groome et al., 1996[▼](#)). The decrease in FSH levels in the mid follicular phase secures the selection of a single dominant follicle (van Santbrink.1995[▼](#)), which becomes less dependent on FSH and continues to grow. Remaining follicles from the recruited cohort cease to mature and undergo atresia.

### ***The LH Surge***

Rising levels of  $E_2$ , produced by the granulosa cells of the pre ovulatory follicle eventually result in a switch from negative feedback, to positive feedback effect resulting in a rapid rise in LH release and the so-called LH surge. The initial onset of the LH surge (not the peak level which is reached 10-12 hours before ovulation) induces the ovulation about 34-36 hours later (Hoff et al., 1983[▼](#)). The mean duration of the LH surge is 48 hours (Hoff et al., 1983[▼](#)). The LH surge initiates multiple events such as the commencement of oocyte meiotic maturation, granulosa cell luteinization and corpus luteum formation as well as the rupture of the dominant follicle allowing for the expulsion of the oocyte (i.e. ovulation) (Conti et al., 1998[▼](#); Richards et al., 1998[▼](#)).

### ***The luteal phase***

Besides triggering of ovulation, the LH surge induces the formation of the corpus luteum. An adequate amplitude and duration of the LH surge are essential for this process (Chandrasekher et al., 1994[▼](#)). Both granulosa cells and theca cells undergo several changes to form the corpus luteum. The wall of the follicle collapses, and capillaries invade the developing corpus luteum probably under the influence of angiogenic and mitogenic factors (McCracken et al., 1999[▼](#)). The differentiated granulosa cells in the corpus luteum will produce progesterone (P) in increasing amounts (Richards et al., 1998[▼](#)),  $E_2$  (Sasano and Suzuki 1997[▼](#)) and Inhibin A (Laven and Fauser 2004[▼](#)).

The maintenance of the corpus luteum is dependent on circulating LH. Several reports confirm that suppression of LH support for 72 hours results in irreversible loss of luteal structure and function (Dubourdieu et al., 1991[▼](#)), but LH or hCG (not FSH) replacement sustains luteal structure and function (Collins et al., 1986[▼](#)). Although the frequency of LH pulses declines during the luteal phase (Ellinwood et al., 1984[▼](#)), prevention of this phenomenon (via pulsatile GnRH infusion or LH injections (Hutchison et al., 1986[▼](#)) does not prevent the luteolysis. The decreasing luteal sensitivity to gonadotrophin could be a critical factor in timely luteolysis near the end of the menstrual cycle (Zelevnik and Little-Ihrig 1990[▼](#)).

Progesterone triggers the endometrial glands to proliferate and become ready for implantation of an embryo. In case an embryo implants and starts to secrete hCG, the corpus luteum is rescued and luteolysis is prevented (Webley et al., 1991[▼](#)). P continues to be secreted by the corpus luteum to maintain pregnancy until the placenta assumes this function, i.e., the luteo-placental shift (McCracken et al., 1999[▼](#)). In an infertile cycle luteolysis is initiated after 12 days and P and Inhibin A levels decrease resulting in menstruation. As a result of the reduced negative feedback effect GnRH pulse frequency will increase and subsequently pituitary FSH production will increase again: the inter-cycle rise.

### ***The endometrium***

In the granulosa cells of growing follicles, cytochrome P<sub>450</sub> aromatase converts androgens into E<sub>2</sub> (Hillier 1994[▼](#)). The different cyclical changes observed in glandular epithelial cells and stromal cells in the endometrium of the uterus are mainly controlled by E<sub>2</sub> and P (Chabbert et al., 1998[▼](#)). Three distinct phases of the development of the endometrium are described during the menstrual cycle: the proliferative phase, corresponding to the follicular phase of the ovary, the secretory phase, corresponding to the luteal phase of the ovary; and the menstrual phase. The morphological and functional maturation of the endometrium is mediated by the specific intracellular receptors for estradiol and progesterone (Lessey et al., 1988[▼](#)). During the secretory phase, a short specific period of uterine receptivity toward embryonic implantation is designated as the “implantation window” (Harper 1992[▼](#)) (see Fig 2).

## **Ovarian hyperstimulation for in vitro fertilization.**

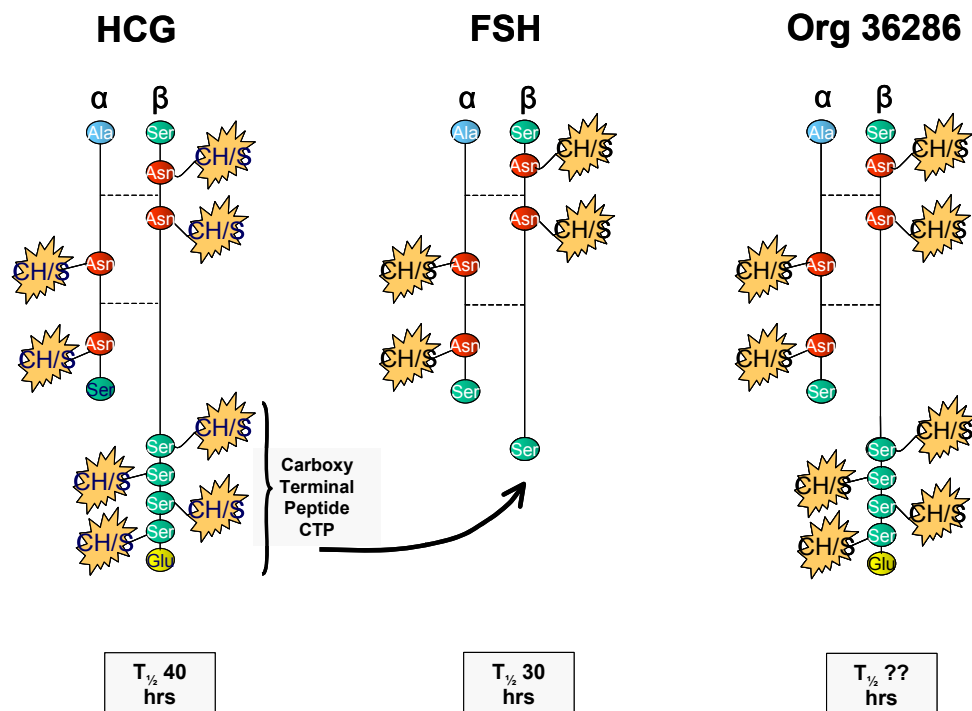
### ***The follicular phase***

In the first years of IVF, the treatment was performed in the unstimulated cycle. Preovulatory oocytes were aspirated laparoscopically soon after the beginning of the spontaneous mid-cycle surge of LH. The LH surge was identified by assaying 3-hourly samples of urine, and measurements of estrogens in 24-hour samples were used to assess follicular growth (Edwards et al., 1980[▼](#)). The successful ongoing pregnancies resulted from oocytes, which were aspirated from their follicles 24 hours or longer after the LH surge began. The oocyte retrieved was fertilized with the husbands' sperm and an embryo was transferred to the uterine cavity 2 days after the oocyte pick up. As only one oocyte was available, the success rates were very low.

In order to increase the chance of obtaining embryos of suitable quality for transfer, ovarian stimulation was introduced. At first patients were treated with oral clomiphene

citrate resulting in 3-4 follicles (Hoult et al., 1981<sup>▼</sup>). Subsequently, human menopausal gonadotrophin (hMG) was introduced (Mettler et al., 1982<sup>▼</sup>). This preparation contains both FSH and LH extracted from urine collected from post-menopausal women. The hMG was injected intramuscularly every day starting on cycle day 2 or 3 until the criteria for final oocyte maturation were met, and the oocyte pick up could be planned. Ovarian hyperstimulation resulted in greater numbers of oocytes and therefore embryos and pregnancy rates improved (Trotnow et al., 1985<sup>▼</sup>).

Subsequently, preparations that were more purified, containing less contaminating non-active proteins, became available. Highly purified products are associated with fewer hypersensitive reactions, and less painful subcutaneous administration. A meta-analysis demonstrated that in IVF cycles the use of FSH is associated with significantly higher clinical pregnancy rates than that of hMG (Daya 2000<sup>▼</sup>). With the growth of IVF, an increasing amount of gonadotrophin preparations became needed and the supply of postmenopausal urine became insufficient. Furthermore, there was a growing concern regarding the risks of the urine contaminants. Therefore, a new method of manufacturing FSH was developed, using the recombinant DNA technique. Recombinant FSH (rFSH) is produced by using Chinese Hamster Ovary cells transfected with expression vectors carrying human cDNAs encoding the common  $\alpha$  gonadotrophin subunit with the FSH



**Figure 3.** Schematic representation of the design of ORG 36286 (adapted from slides kindly provided by Organon N.V.)

specific  $\beta$ -subunit (Recombinant Human FSH Product Development Group 1998[▼](#)). With this technique it became possible to produce the human FSH molecule in the laboratory with constant quality and constant bioactivity. The first pregnancies with rFSH were published in 1992 (Devroey et al., 1992[▼](#); Donderwinkel et al., 1992[▼](#); Germond et al., 1992[▼](#)). The latest step in the development of the gonadotrophins is the new FSH-CTP molecule (ORG 36286). This is a modified human FSH molecule. All gonadotrophins are glycoproteins consisting of identical  $\alpha$  chain and a hormone specific  $\beta$  chain (Moyle et al., 1998[▼](#)). The half-life of FSH is 32 ( $\pm$  10 hour) (Mannaerts et al., 1993[▼](#)) whereas that of human chorionic gonadotrophin (hCG) is 31-56 hours (Damewood et al., 1989[▼](#)). The Carboxy terminal peptide (CTP) at the  $\beta$  chain of hCG is responsible for this longer half life. To extend the half-life of FSH, in order to create a long-acting follicle stimulant, the CTP was added to the  $\beta$  chain of FSH (see Fig. 3). This indeed resulted in a longer half-life (Bouloux et al., 2001[▼](#); Fares et al., 1992[▼](#)), and FSH CTP is currently undergoing clinical testing.

The major goal of the use of FSH in IVF treatments is to induce the development and growth of multiple follicles. By increasing the FSH levels above the FSH threshold for a extended period of time in the follicular phase, more than one follicle will gain dominance and continue to grow (Schipper et al., 1998b[▼](#)). One of the challenges of the current IVF practice remains the optimisation of the treatment protocols. While much data are available regarding the prediction of poor response (Bancsi et al., 2002[▼](#)), predicting the individual response to a certain FSH dose remains a challenge. Age, BMI, early follicular phase FSH levels ovarian volume and amount of small follicles present in the ovaries in the early follicular phase are all parameters which influence the response (Ng et al., 2000[▼](#)), but there is no direct relationship. A prediction model was made and subsequently validated in the same centre (Popovic-Todorovic et al., 2003a[▼](#); Popovic-Todorovic et al 2003b[▼](#)). In this study 77% of the patients presented with an 'appropriate response' defined as 5-14 oocytes. In 23% the response was either higher or lower than expected, indicating that the model is not completely reliable. Moreover, until now, an optimal number of oocytes has not been defined. Therefore, there is no international agreement on the individual daily dose of FSH. Furthermore, some clinicians adapt the dose after some days of stimulation, while others use fixed doses. In addition, each IVF clinic uses its own definition of poor response and high response. The cut off points for cancellations of the treatment cycle on either too few or too many follicles are also not defined. The lack of agreement on these points results in difficulties on the interpretation of the literature.

### ***Prevention of the LH surge***

In the early days of ovarian hyperstimulation for IVF, a premature LH surge and the subsequent premature luteinization were the major problems. Indeed, 20-40% of all IVF cycles were cancelled before the oocyte pick up could be performed (Hughes et al., 1992[▼](#)). The introduction of GnRH agonist treatment more than 20 years ago to prevent



the LH surge was a major step forward (Porter et al., 1984[▼](#)). From the mid 1980's, the use of GnRH agonists became the routine practice worldwide (Hughes et al., 1992[▼](#)). The most commonly applied regimen is the so-called 'long protocol' in which treatment with a GnRH agonist is initiated either in the mid luteal phase, or on day 1 of the cycle. After the initiation of a GnRH agonist, the pituitary gland will be stimulated to produce and release both extended amounts of LH and FSH. This is the so-called 'flare up' effect. If the GnRH agonist is continued for a extended period of time, LH and FSH release diminish and finally virtually disappear. The GnRH receptors on the pituitary gland become less sensitive and eventually disappear by internalization. This process is referred to as the downregulation of the pituitary gland (Brogden et al., 1990[▼](#)). In about 9.3 % of the women treated with GnRH agonist before IVF, functional cysts will develop (Qublan et al., 2005[▼](#)). This could be due to the initial high FSH levels. These cysts are associated with lower outcome parameters (Qublan et al., 2005[▼](#)). Most treatment protocols for IVF start the ovarian hyperstimulation after downregulation has been confirmed. With this regimen only about 1% of the cycles has to be cancelled due to premature ovulation (Smitz et al., 1992b[▼](#)).

Although the pregnancy rates improved considerably (Hughes et al., 1992[▼](#)), some disadvantages associated with the use of the GnRH agonist became apparent. First of all, the IVF treatment became more complex. The period of time in which the women use daily injections became much longer, the amount of FSH that has to be used became higher (Smitz et al., 1992b[▼](#)), and the side effects were worse. These side effects are due to the transient hypo-estrogenic status caused by the pituitary downregulation. Finally, after the cessation of the GnRH agonist it takes at least two to three weeks for endogenous LH and FSH release to be restored (Donderwinkel et al., 1993[▼](#)).

Initial attempts to develop a GnRH antagonist (which could block the GnRH receptors and immediately prevent a LH surge), all failed, as the substances could not be administered without severe local reactions. In 1999, GnRH antagonists became clinically available (The European and Middle East Orgalutran Study Group 2001[▼](#)). These antagonists are competitive blockers of the GnRH receptors (Oberye et al., 1999[▼](#)). Initial studies demonstrated that GnRH antagonists were effective in preventing the premature LH surge and subsequent ovulation. The cancellation rate for premature ovulation is only 1-2% after the use of GnRH antagonist (Al Inany and Aboulghar 2002[▼](#)), which is comparable with the cancellation rate after the use of a GnRH agonist (Smitz et al., 1992b[▼](#)). Two approaches to use the GnRH antagonist have been developed: either fixed or flexible timing of the commencement (Al Inany et al., 2005b[▼](#)). In the fixed protocol, the initiation of the GnRH antagonist is always on the same stimulation day, for example day 6, while in the flexible protocol, the start of the GnRH antagonist is depending on the magnitude of ovarian response (i.e. the diameter of the leading follicle). It seems more reasonable only to inhibit LH in case there is an imminent LH surge, which cannot be expected when all follicles are still relatively small. However, a fixed start is easier, since in the flexible start ultrasound examination has to take place before the initiation of the GnRH antagonist and



there is a rather wide variation in the stimulation day on which the largest follicle is 14-15 mm. A recent meta-analysis showed that there was no statistically significant difference in pregnancy rate per woman randomized, although there was a trend towards a higher pregnancy rate with the fixed protocol, especially with delayed administration beyond day 8 (Al Inany et al., 2005b[▼](#)). This is probably due to the fact that in some women the antagonist was started too late in the flexible protocols.

The use of a GnRH antagonist has many advantages. The ovarian hyperstimulation can be performed in the natural menstrual cycle, without pituitary downregulation, which makes the duration of the treatment much shorter and the side effects less. Furthermore, without the use of the GnRH agonist it is possible to use a mild stimulation protocol, which aims at the retrieval of less oocytes than the conventional protocols (de Jong et al., 2000[▼](#)). Therefore, the introduction of the GnRH antagonist seems an important step in the direction of more individualized treatment protocols which can finally be more patient friendly and safer (Macklon and Fauser 2003[▼](#)).

### ***Final oocyte maturation***

As the LH surge is prevented during ovarian hyperstimulation for IVF, the final oocyte maturation is routinely performed by the use of a hCG bolus, 32 – 36 hours before the oocyte retrieval. This is possible since hCG binds to the LH receptors (Pierce and Parsons 1981[▼](#)). The granulosa cells will lose their tight junction and the oocytes will undergo their final maturation step from metaphase 1 into metaphase 2. The hCG employed is extracted from urine from pregnant women. Only recently, recombinant hCG has become available (The European Recombinant Human Chorionic Gonadotrophin Study Group 2000[▼](#)). In addition, recombinant LH has been developed, but is not yet available on the market in the dosage needed for final oocyte maturation. A very recent meta-analysis showed no differences in pregnancy rates after the use of either urinary or recombinant hCG (Al Inany et al., 2005a[▼](#)). The major difference between the LH surge (in the natural cycle) and the hCG bolus (routinely applied in IVF) is the duration. As the LH surge will last about 48 hours (Hoff et al., 1983[▼](#)), 10.000 IU of hCG are cleared from the circulation 8 days after injection. This is due to the fact that the half-life of LH is only 20 minutes, while the half-life of hCG is about 31-56 hours (Damewood et al., 1989[▼](#)). Moreover, the release of LH from the pituitary gland is influenced by negative feedback systems. A problem associated with the use of the hCG bolus is the risk of the development of an ovarian hyperstimulation syndrome (OHSS). The prolonged action of the hCG could be responsible for this, although the exact mechanisms causing OHSS are still unclear. Some authors have suggested that reducing the hCG dose could diminish the risk of developing OHSS (Delvigne and Rozenberg 2002[▼](#)), but the data are contradictory (Schmidt et al., 2004[▼](#)). However, concern about a possible negative influence of the use of hCG has led to the development of new strategies for final oocyte maturation. The introduction of the GnRH antagonists made it possible to use the flare up effect which occurs after the administration

of a GnRH agonist (Gonen et al., 1990[▼](#)). A bolus of a GnRH agonist will initiate a release of endogenous FSH and LH, which can trigger the final oocyte maturation. It is well documented that this is effective indeed (Fauser et al., 2002[▼](#)). Mature oocytes will be recovered and the fertilization rates are comparable with the rates after the use of hCG. In addition, some authors have shown that the risk of developing OHSS is lower after the use of a GnRH agonist for final oocyte maturation (Tay 2002[▼](#)). Unfortunately, two recent studies showed that the pregnancy rates are dramatically reduced with this regimen (Humaidan et al., 2005[▼](#); Kolibianakis et al., 2005[▼](#)). This is probably due to the extremely short duration of the endogenous LH surge after a GnRH agonist.

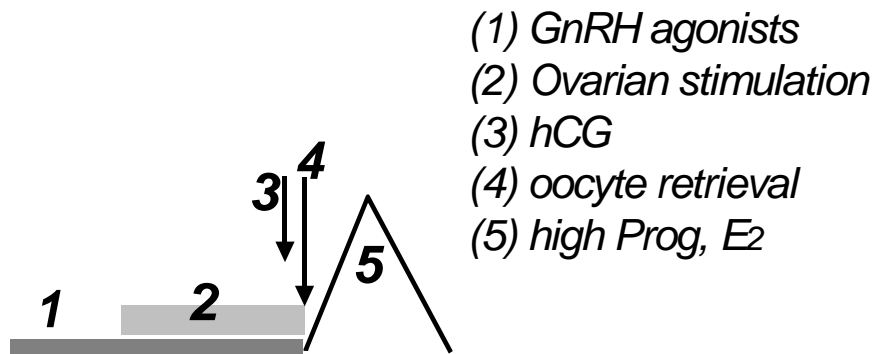
### ***The luteal phase***

Since the early days of IVF it has been suggested that the luteal phase after ovarian stimulation becomes shorter and insufficient resulting in lower pregnancy rates (Smitz et al., 1988[▼](#)). Therefore luteal support has been routinely used since the late 1980s. Many different protocols have been described and apparently two different approaches can be distinguished. Either the punctured follicles which form the corpora lutea can be supported to produce sufficient P levels themselves, during a sufficient period of time, or P itself can be administered. For the production of steroids, the luteinized granulosa cells of the corpora lutea need stimulation by LH, but LH levels are low in patients who underwent ovarian hyperstimulation for IVF (see below). As in the replacement of the LH surge with a hCG bolus, in the luteal phase, the corpora lutea can also be supported with repeated injections of hCG. Again, this is known to increase the risk of the development of OHSS, and therefore alternative strategies have been introduced. The administration of P itself has been established for many years. Different doses, durations and types of treatments are used, but the most effective dose, duration or type of treatment remains controversial. In a recent meta-analysis (Pritts and Atwood 2002[▼](#)) it was stated that vaginal gel preparations seem no better or worse than micronized oral P used in a vaginal administration. Intramuscular doses seem no better or worse if 25 or 100 mg are used, and natural P appears no better or worse than 17-hydroxy-P in increasing pregnancy rates. However, it is worth noting that most of these comparisons are from small, individual studies, and thus the power to detect clinically significant differences is low. Intramuscular P is more effective than vaginal P. However, intramuscular injections are not only painful, but can also lead to inflammation and even sterile abscess formation at the injection site (Tavaniotou et al., 2000[▼](#)). Severe allergic reactions to the oil used as a vehicle for P injections have also been reported. Vaginal application of P has also led to some minor side effects such as vaginal discharge and irritation (Kimzey et al., 1991[▼](#)). Some evidence also exists that adding E<sub>2</sub> to P may increase the implantation rate, but not the pregnancy rate. Finally, the duration of the luteal support varies widely. Some clinicians continue P for 13 days (Artini et al., 1995[▼](#); Perino et al., 1997[▼](#)), while others use it routinely until 12 weeks of gestation (Abate et al., 1999[▼](#); Check et al., 1991[▼](#)). In one study, it was shown

that P levels were higher in women with IVF pregnancies (due to the multiple corpora lutea) compared to women with spontaneous pregnancies. This was the case both in women who continued luteal support and in women who stopped luteal support 2 weeks after gestation. (Costea et al., 2000 [v](#)). These findings suggest strongly that there is no need for continuing the medication.

A number of studies have focused on the mechanisms underlying the early luteolysis after ovarian hyperstimulation. In studies comparing luteal phase aspects with and without luteal phase support, it was shown that in non-supplemented patients, the early luteal phase P production was clearly supraphysiologic, as a result of the presence of the multiple corpora lutea. However, P levels decrease very rapidly to low levels at day 6 after oocyte pick up already. This indicates that the corpora lutea are only supported in the early luteal phase, but not in the mid and late luteal phase, which is crucial for the implantation. In the early luteal phase this support comes from the hCG bolus administered 35 hours before the oocyte pick up, whereas in the mid and late luteal phase this exogenous hCG is gone. It is clear that in this phase, the corpora lutea are not supported anymore, as endogenous hCG

## Luteal / endometrial dysfunction following IVF



**Figure 4.** Schematic representation of the 5 possible mechanisms behind low LH levels and early luteolysis luteal phase after ovarian hyperstimulation for IVF

or LH is (still) lacking.

There are various theories regarding the low LH levels in the luteal phase after ovarian hyperstimulation for IVF (see figure 4). (1) It has been known for a long time that after the cessation of the GnRH agonist in the downregulated patient, the recovery of the LH levels

takes at least 2-3 weeks. As the GnRH agonist is only stopped 2 days before the oocyte pick up it is clear that the LH levels are still low in the luteal phase. This problem could theoretically be solved in patients who are not co-treated with a GnRH agonist. Indeed, after the introduction of the GnRH antagonist it was suggested that luteal support would be no longer needed. This assumption was based on pharmacokinetic studies performed with GnRH antagonists. These studies showed that LH and FSH production recovered within 24 hours after the cessation of the antagonist. (2) In a study from our group, it was shown in volunteers that multifollicular growth by itself had an effect on the duration of the luteal phase (Hohmann et al., 2001[▼](#)). It remains unclear what the exact mechanism behind this effect would be. (3) It has also been suggested that the hCG bolus itself would have a negative feedback effect on the pituitary LH synthesis and release. However, in a recent study this effect could not be confirmed in normo-ovulatory women (Tavaniotou and Devroey 2003[▼](#)). (4) The removal of the P producing theca cells during the oocyte pick up could also influence the capacity of P production by the corpora lutea (Garcia et al., 1981[▼](#); Vargyas et al., 1986[▼](#)). (5) Finally, low LH levels in the luteal phase after ovarian hyperstimulation could be the result of supraphysiologic steroid levels. With the development of multiple follicles, E<sub>2</sub> levels are clearly supraphysiologic in the late follicular and early luteal phase, whereas the P levels are supraphysiologic in the early to mid luteal phase. These levels could have a negative effect on the pituitary gland, suppressing the LH release.

## **Objectives of this thesis**

The first part of this thesis is focused on one of the clinical studies performed with the new long acting molecule FSH-CTP (ORG 36286). The second patient, treated in this phase 2 dose finding study became pregnant. Details regarding the treatment of this patient were described in paragraph 2.1. The results of the dose finding study itself have been described in paragraph 2.2.

The second part of this thesis focuses on the luteal phase characteristics in patients undergoing ovarian hyperstimulation for IVF. In GnRH agonist co-treated IVF patients, we have tried to improve luteal phase characteristics in cycles without luteal support. As mentioned above, LH levels remain low for a longer period of time after cessation of the GnRH agonist. Therefore, the GnRH agonist was withheld from the early follicular phase. Clinical and endocrine characteristics are described in paragraph 3.1. Subsequently, we studied luteal phase characteristics in IVF patients co-treated with a GnRH antagonist. Clinical and endocrine characteristics of patients participating in this study are described in paragraph 3.2. Data from the literature, combined with our own findings, strongly suggest that especially the supraphysiologic steroid levels, due to multi follicular growth, are the most important factors inducing premature luteolysis in patients undergoing ovarian

hyperstimulation for IVF. This model was tested in normo-ovulatory volunteers treated with high dosages of  $E_2$ , P or both. The results of this study are described in paragraph 3.3.



## **Chapter 2:**

### **Follicular phase aspects of IVF treatments**

## 2.1 First live birth after ovarian stimulation using a chimeric long-acting human recombinant follicle-stimulating hormone (FSH) agonist (recFSH-CTP) for in vitro fertilization

### Introduction

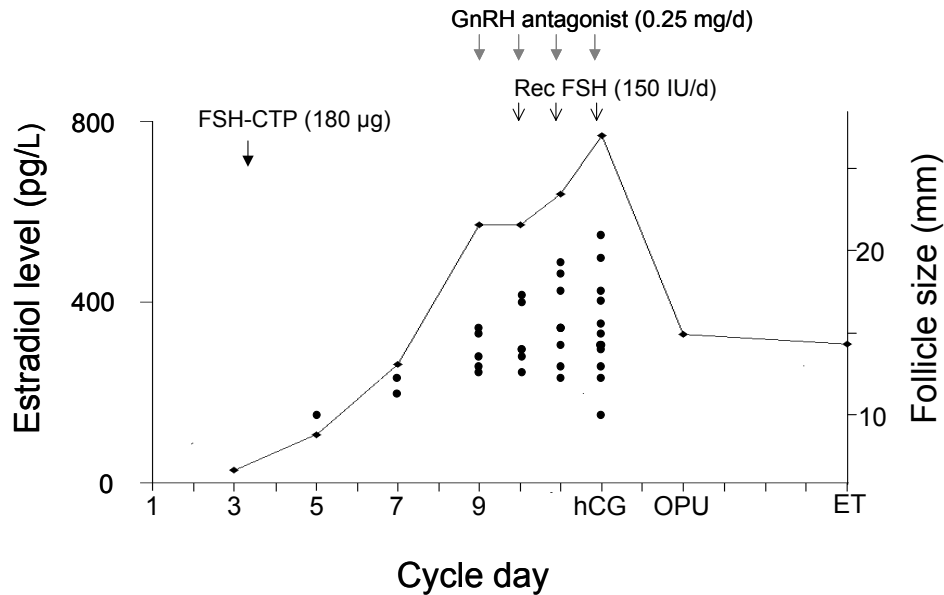
The gonadotropin/thyrotropin hormone family is characterized by a heterodimeric structure consisting of a common  $\alpha$ -subunit and a hormone-specific  $\beta$ -subunit (Pierce and Parsons 1981[▼](#)). The  $\beta$ -subunit of the pregnancy hormone hCG is distinctly different from the others because of an extension at the carboxy-terminal end, i.e., a C-terminal peptide (CTP) with four O-linked oligosaccharides. Analysis of the  $\beta$ -hCG coding sequence suggests that this extension is due to the loss of the termination codon of the ancestral  $\beta$  LH gene (Fiddes and Goodman 1980[▼](#)). Both hCG and LH bind to the same receptor and exhibit comparable bioactivity in vitro (Keutmann et al., 1987[▼](#)). The CTP extension of the  $\beta$ -subunit of hCG has been shown to be responsible for the reduced clearance resulting in a major enhancement of in vivo bioactivity (Damewood et al., 1989[▼](#)).

FSH is essential for follicle development and is applied widely in clinical practice to stimulate ovarian function (Fauser and van Heusden 1997[▼](#)). The stable transfection of the common  $\alpha$ - and the  $\beta$ -FSH-subunit into Chinese hamster ovary cells has allowed for the development (Keene et al., 1989[▼](#)) and clinical introduction (Recombinant Human FSH Product Development Group 1998[▼](#)) of human recombinant FSH (recFSH). The first pregnancies after the use of recFSH for ovulation induction in anovulatory women (Donderwinkel et al., 1992[▼](#)) and for ovarian stimulation for IVF (Devroey et al., 1992[▼](#)) were reported a decade ago. The relatively short half-life of FSH preparations ( $32 \pm 12$  hours) (Mannaerts et al., 1993[▼](#)) requires daily injections, which cause considerable discomfort for the patient. In an attempt to create a long-acting FSH agonist preparation, chimeric genes containing the sequence encoding the CTP of  $\beta$ -hCG fused with  $\beta$ -FSH were constructed (Fares et al., 1992[▼](#)). First human exposure showed that recFSH-CTP could be administered safely in hypogonadal males (Bouloux et al., 2001[▼](#)) and showed an extended half-life of 95 hours (Duijkers et al., 2002[▼](#)).

### Case report

A 32-year-old woman presented with a 7-year history of primary infertility. After a diagnosis of cervical hostility, she underwent 6 cycles of IUI without success and subsequently began IVF treatment. The first two IVF cycles ended in total fertilization failure. Before a subsequent IVF/intracytoplasmic sperm injection (ICSI) cycle, she was enrolled in a multicenter, phase 2, dose-finding study for recFSH-CTP (corifollitropin alpha; NV





**Figure 1.** Estradiol serum concentrations and number and size of dominant follicles during ovarian stimulation for IVF using a recFSH-CTP, recFSH, and GnRH antagonist combination of medication on the day of hCG (hCG), of oocyte retrieval (OPU), and of ET.

Organon, Oss, The Netherlands). The study was approved by the local ethics review committee, and written informed consent was obtained.

Ovarian hyperstimulation was initiated on day 3 of the spontaneous menstrual cycle with a single SC injection of 180 µg of recFSH-CTP. Normal multiple dominant follicle development and rising serum E<sub>2</sub> levels could be observed (Fig. 1). On cycle day 9, the largest follicle exceeded 14 mm in diameter, and cotreatment was initiated with the GnRH antagonist ganirelix (Orgalutran, NV Organon) 0.25 mg/day SC for the prevention of an early rise in serum LH concentrations and subsequent chances for premature luteinization. According to the protocol, ovarian stimulation was continued from cycle day 10 (7 days after the recFSH-CTP injection) onward, with daily SC injections of 150 IU recFSH (Puregon, NV Organon). On cycle day 12, 10,000 IU hCG (Pregnyl, NV Organon) was administered SC as a single injection.

Twelve oocytes were subsequently retrieved from a total of 14 follicles exceeding 10 mm. Ten oocytes were fertilized in vitro by ICSI, and two embryos were subsequently transferred after 3 days of culture. Luteal support was provided using 600 mg/day of micronized P (Progestan, NV Organon) intravaginally. Two remaining good-quality embryos were cryopreserved. The pregnancy test was positive 2 weeks after ET, and

ultrasound investigation revealed an intact, intrauterine, singleton pregnancy after 12 weeks. A healthy child was spontaneously delivered at term. Subsequently, more patients have entered the study and several pregnancies are currently ongoing.

## Discussion

The current case report suggests that early follicular phase administration of recFSH-CTP -the first long-acting FSH agonist- can effectively and safely circumvent the daily injections that are required for ovarian stimulation in IVF. A single injection provided sufficient ovarian stimulation to enable multifollicular growth over a period of 7 days. The ovarian response assessed in terms of follicular development and serum E<sub>2</sub> levels is shown in Figure 1. The E<sub>2</sub> concentrations were comparable to those measured after daily injections of recFSH using either GnRH agonist (Devroey et al., 1994[▼](#)) or antagonist (de Jong et al., 2001[▼](#)) cotreatment.

A recent pharmacokinetic study using recFSH-CTP established that maximum FSH serum levels were reached 36–48 hours after injection along with a terminal half-life of 60–75 hours (Duijkers et al., 2002[▼](#)), which is 2–3 times longer compared with recFSH (Mannaerts et al., 1993[▼](#)). For this reason, a 7-day interval was chosen in an attempt to improve patient convenience compared with currently available daily injections. Considering the long half-life of recFSH-CTP, a second injection on cycle day 9 may induce unacceptable risks for ovarian hyperstimulation syndrome. It was therefore decided to continue with daily recFSH injections. Indeed, only 3 days of recFSH were required to reach the suitable number of preovulatory follicles for induction of oocyte maturation and subsequent oocyte retrieval. The current case report describes the first reported pregnancy observed in a multicenter, feasibility trial. This and subsequent studies should reveal the optimal regimen for recFSH-CTP administration for ovarian stimulation.

## 2.2 Induction of multiple follicular development by a single dose of long-acting recombinant follicle-stimulating hormone (FSH-CTP, Corifollitropin Alfa) for controlled ovarian stimulation before *in vitro* fertilization

### Introduction

Clinical protocols for induction of multifollicular development in women undergoing conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) commonly rely on daily FSH injections. The availability of a longer-acting FSH preparation with a comparable biopotency might allow for the development of new treatment regimens requiring fewer injections. Long-acting FSH may be created by additional glycosylation of the FSH molecule (Perlman et al., 2003[▼](#)) or by coupling the carboxy-terminal part (CTP) of the  $\beta$ -subunit of human chorionic gonadotropin (hCG) to the FSH  $\beta$ -subunit (Fares et al., 2003[▼](#)). The very first report on the design of a long-acting FSH agonist was by Boime and coworkers (Klein et al., 2003[▼](#); Fares et al., 1992[▼](#)), who used site-directed mutagenesis and gene transfer techniques to develop FSH-CTP, which is produced and secreted by a Chinese hamster ovary cell line and contains four N-linked carbohydrate chains ( $\alpha$ 52,  $\alpha$ 78,  $\beta$ 7, and  $\beta$ 24) and four O-linked carbohydrate chains at the CTP ( $\beta$ 115,  $\beta$ 121,  $\beta$ 126, and  $\beta$ 132), the latter causing a 3- to 4-fold increased *in vivo* half-life as compared with wild-type recombinant FSH (rFSH).

Preclinical research has indicated that FSH-CTP has an *in vitro* pharmacological activity comparable to rFSH and an anticipated half-life that is 2- to 3-fold longer compared with rFSH. Because of its long half-life, standardization of FSH-CTP by means of the classical Steelman-Pohley bioassay (Steelman and Pohley 1953[▼](#)) does not provide a reliable estimate of its *in vivo* bioactivity from a pharmacological or clinical perspective. Therefore, dosages of pure recombinant FSH-CTP are expressed in mass ( $\mu$ g) instead of international units.

A general concern for all therapeutic biopharmaceuticals, especially for designed analogs with deviating amino acid sequence or carbohydrate site chains (Schellekens 2002[▼](#)), is their potential immunogenicity induced by repeated injections or long-term treatment (Bouloux et al., 2001[▼](#)). Therefore, the first human study of FSH-CTP was performed in hypogonadotropic hypogonadal male volunteers who received four single sc injections of FSH-CTP at 4-wk intervals (Bouloux et al., 2001[▼](#)). Repeated FSH-CTP administration appeared to be safe and well tolerated and did not give rise to antibody formation. In these hypogonadotropic men, serum FSH-CTP levels peaked on average 46 h after injection, whereas the average elimination half-life was 95 h. In a second FSH-CTP study in healthy pituitary-suppressed female volunteers, a single injection of 120  $\mu$ g FSH-CTP induced multiple follicle growth (Duijkers et al., 2002[▼](#)) comparable to that induced by daily 150 IU rFSH for 7 d using the same study model (Voortman et al., 2000[▼](#)).

Pharmacokinetic analysis revealed that peak serum FSH-CTP concentrations were reached at about approximately 36 h after injection and that the terminal half-life was between 60 and 75 h.

During controlled ovarian stimulation, daily administration of exogenous FSH overrides the normal selection process of a single dominant follicle by fulfilling the FSH threshold window requirements for multiple precursor follicles (Fauser and Devroey 2003[▼](#)). When a single dose of long-acting FSH-CTP is given at a suprathreshold dose, multiple follicular development will be induced that will persist as long as serum FSH-CTP levels remain above the threshold requirements of these follicles. In case FSH-CTP levels decline below the threshold, FSH-sensitive follicles cease to develop and become atretic. During stimulation, this follicular atresia could be prevented by switching in a timely manner to the treatment with daily rFSH. Very recently, the first successful treatment of a patient with a 7-yr history of primary infertility was reported after treatment with a single dose of 180 µg FSH-CTP followed by three injections of 150 IU rFSH (Beckers et al., 2003a[▼](#)).

The current study was performed to explore a new treatment regimen for controlled ovarian stimulation using a single dose of long-acting FSH-CTP to support 7 d of multiple follicular growth followed by the daily administration of rFSH to complete the stimulation cycle up to the day of hCG administration. For that purpose, subjects scheduled for IVF or ICSI received either 120, 180, or 240 µg FSH-CTP followed by daily injections of 150 IU rFSH. The primary endpoint of this trial was the total dose of rFSH, as it was assumed that increasing doses of FSH-CTP would require less rFSH to reach the same criteria of hCG. Apart from an evaluation of clinical outcome parameters, the number and size of follicles as well as hormonal responses were compared with those of subjects who received daily rFSH administration.

## **Materials and Methods**

### ***Subjects***

In total, 99 subjects were randomized: 25 subjects to the 120-µg FSH-CTP group, 25 subjects to the 180-µg FSH-CTP group, 25 subjects to the 240-µg FSH-CTP group, and 24 subjects to the 150-IU rFSH group. All subjects underwent ovarian stimulation for conventional IVF or ICSI. Subjects were between 18 and 39 yr of age and had a regular menstrual cycle (24–35 d) and normal body weight (body mass index 18–29 kg/m<sup>2</sup>). This study was approved by the local ethical committees of all three participating centers and is in agreement with the Declaration of Helsinki for Medical Research Involving Human Subjects.

### **Study design**

This study was an open-label randomized four-arm trial. Subjects in the FSH-CTP groups started ovarian stimulation on cycle d 2 or 3 with a single sc dose of FSH-CTP (Org 36286, corifollitropin alfa, NV Organon, The Netherlands) of 120, 180, or 240 µg followed 1 wk later (treatment d 8) with a fixed daily sc dose of 150 IU rFSH (rFSH/Puregon/Follistim, NV Organon) up to and including the day of hCG. In the control group, subjects started on cycle d 2 or 3 with a fixed daily sc dose of 150 IU rFSH up to and including the day of hCG. To prevent premature LH surges, a GnRH antagonist (ganirelix; 0.25 mg in 0.5 ml daily, Orgalutran/Antagon, NV Organon) was administered sc starting on the day that the leading follicle had reached 14 mm. When at least three follicles greater than or equal to 17 mm were observed by transvaginal sonography, hCG (10,000 IU Pregnyl, NV Organon) was administered sc for the induction of final oocyte maturation. Approximately 30–36 h later, oocyte retrieval followed by conventional IVF or ICSI was performed. At embryo transfer (ET), 2–5 d after oocyte pick-up, no more than three embryos were replaced. All subjects received luteal-phase support by means of vaginal micronized progesterone (600 mg/d) or im progesterone (50 mg/d) starting on the day of embryo transfer, at the latest.

### **Assessments**

Before the start of ovarian stimulation, pregnancy was excluded by means of an hCG test. A blood sample was taken for hormone assessments, and ultrasound was performed. The subject returned to the clinic for transvaginal ultrasound and blood sampling on d 3, 5, and 7 and daily thereafter up to and including the day of hCG. Blood sampling was performed before ganirelix and FSH administration. Additional blood samples of all subjects were taken on the day of oocyte pick-up, the day of ET, and at 2 wk after ET.

Serum FSH-CTP levels were determined by enzyme immunoassay (EIA) with a coefficient of variation (CV) of less than 20% and a lowest detection limit of 0.079 ng/ml. Antibodies against FSH-CTP were assessed using an RIA based on the formation of an immune complex between a specific antibody and <sup>125</sup>I-labeled FSH-CTP. These samples were assessed with a coefficient of variation of less than 20% [the assays for FSH-CTP and for antibodies against FSH-CTP are also described by Bouloux *et al.* (Bouloux *et al.*, 2001<sup>1</sup>)]. Serum FSH, LH, estradiol (E<sub>2</sub>), and progesterone (P) levels were determined by time-resolved fluoroimmunoassay (AutoDELFIA, Wallac Oy, Finland) with a coefficient of variation of less than 20%. Lowest detection limits were 0.25 IU/liter, 0.6 IU/liter, 13.6 pg/ml, and 0.31 ng/ml for FSH, LH, E<sub>2</sub>, and P, respectively. All measurements were performed at NV Organon using validated assays.

Serum inhibin-B levels were determined by EIA (Oxford Bio-Innovation Ltd., Oxford Shire, UK) with a coefficient of variation of less than 20% and a lowest detection limit of 15.6 pg/ml. The inhibin-B levels were assessed by a central laboratory (AAI, Neu-Ulm, Germany) using a validated assay.

### Statistical methods

The aim of this study was to measure the efficacy and efficiency of the various FSH-CTP doses; thus, the precision of the estimates, rather than the power of the study, was of interest. The primary endpoint of the study was the rFSH dose needed from treatment d 8 onwards, as it was assumed that with higher doses of FSH-CTP less rFSH would be needed to reach the same criteria for hCG administration. With 25 subjects treated in each group, the total dose of rFSH could be estimated with a precision (SE) of approximately 70 IU (based on a SD of 350 IU). This means that a two-sided 95% confidence interval of the

**Table 1.** Subject characteristics and disposition per treatmentgroup

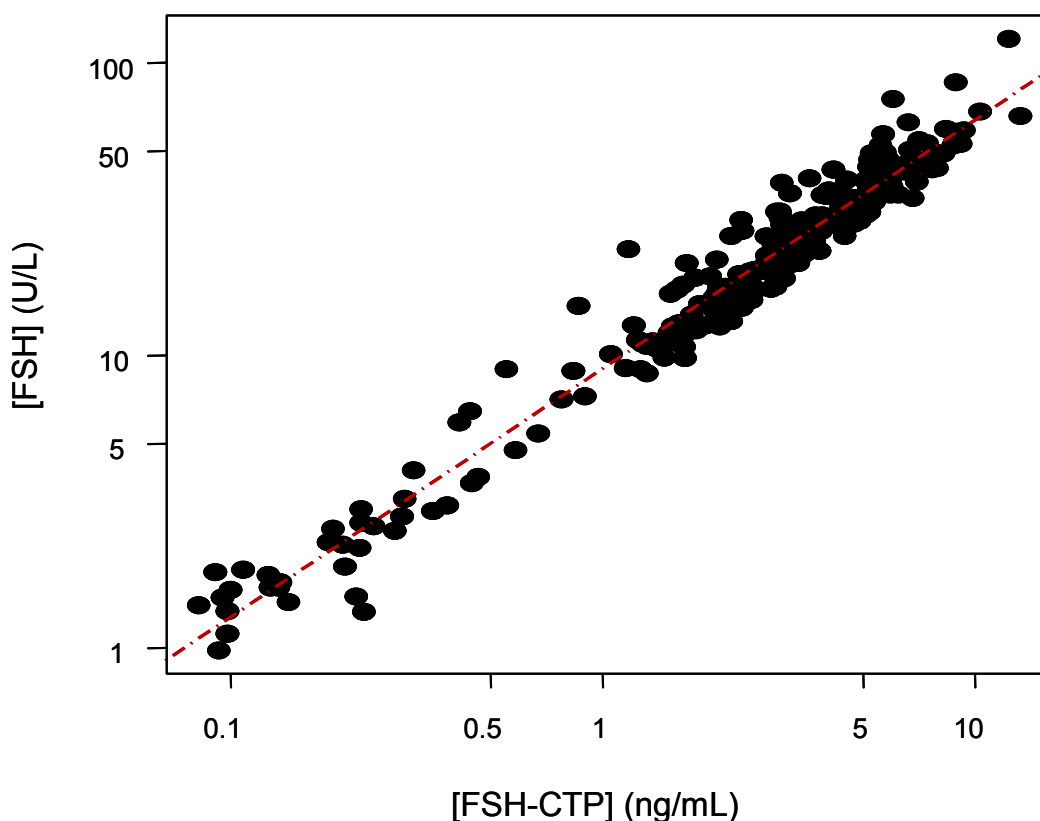
	120 µg	FSH-CTP 180 µg	240 µg	rFSH 150 IU
Age (yrs)	30.4 ± 3.8	31.5 ± 3.8	33.4 ± 4.1	32.1 ± 4.3
BMI (kg/m <sup>2</sup> )	23.2 ± 2.8	22.9 ± 3.5	22.6 ± 2.7	23.4 ± 2.8
Primary infertility (%)	56	46	56	42
Main causes infertility (%)				
Tubal	28	4	32	33
Male	40	42	44	38
Tubal and male	0	17	0	4
Unknown	20	25	20	17
Endometriosis	8	4	0	0
Duration of infertility (yrs)	4.2 ± 3.1	4.9 ± 3.6	5.6 ± 4.3	4.0 ± 3.2
Subjects (N)				
Start stimulation	25	24	25	24
HCG	25	23	23	23
OPU	23	22	23	23
ET	20	21	21	23

total dose of rFSH has a width of 280 IU (*i.e.* mean total dose ± 140 IU). With a daily fixed rFSH dose of 150 IU, this coincides with a width of approximately 2 d for the confidence interval of the mean treatment duration (*i.e.* mean treatment duration ± 1 d) Serum FSH-CTP levels were analyzed using the nonlinear mixed-effects model program NONMEM. A one-compartment model with first-order absorption with the subject's weight as covariate on total serum clearance and volume of distribution gave an accurate description of the

serum FSH-CTP concentrations in time. A fixed value for absorption rate  $k_a$  of  $0.102 \text{ h}^{-1}$  was assumed (as estimated in a previous trial with FSH-CTP), because no information was available in this trial on the absorption phase of the pharmacokinetic profile. For this model, both population (mean) and individual parameter estimates were obtained. Individual estimates of exposure to FSH-CTP ( $AUC$  and  $C_{max}$ ) were calculated from individual serum FSH-CTP levels.

In addition to summary statistics, for continuous parameters such as the duration of stimulation, a treatment-group comparison was performed using ANOVA. If the  $P$  value of the ANOVA model was 0.05, Dunnett's  $t$  test was performed to compare the three FSH-CTP groups with the Puregon group; if the  $P$  value of the ANOVA model was  $>0.05$ , no further comparison between treatment groups was performed. The dose-response

$$\text{Linear Model (y=ax+b)} : \ln(\text{FSH}) = 0.85 \cdot \ln(\text{FSHCTP}) + 2.198$$



**Figure 1.** Correlation plot of serum samples measured by the FSH-CTP immunoassay and the FSH Delfia

relationship of FSH-CTP with respect to the number of follicles was assessed by using a linear regression model with dose as the covariate. The frequency of incidence of premature LH rises was analyzed using the exact  $\chi^2$  method.

## Results

### *Subjects characteristics and disposition*

A total of 98 subjects were randomized and treated: 25 with 120 µg FSH-CTP, 24 with 180 µg FSH-CTP, 25 with 240 µg FSH-CTP, and 24 with daily 150 IU rFSH. Demographic and fertility characteristics at screening and the number of subjects who started treatment and had oocyte pick-up and ET are included in Table 1. There were no differences between the four treatment groups with respect to age ( $P = 0.06$ ), body mass index ( $P = 0.79$ ), incidence of primary infertility ( $P = 0.67$ ), duration of infertility ( $P = 0.22$ ), or cause of infertility ( $P = 0.38$ ).

Four subjects started stimulation but did not receive hCG: two subjects because of an excessive response (180 µg FSH-CTP and 240 µg FSH-CTP, respectively) and two

**Table 2.** Mean ( $\pm$  SD) serum FSH-CTP levels and derived pharmacokinetic parameters

		Dose of FSH-CTP		
		120 µg	180 µg	240 µg
	<i>Units</i>			
Day 3	ng/mL	3.5 $\pm$ 1.2	5.5 $\pm$ 1.8	7.3 $\pm$ 2.2
Day 5	ng/mL	2.3 $\pm$ 0.7	3.6 $\pm$ 1.1	4.8 $\pm$ 1.3
Day 7	ng/mL	1.3 $\pm$ 0.4	2.2 $\pm$ 0.7	2.8 $\pm$ 0.7
Day ET	ng/mL	0.13 $\pm$ 0.05	0.18 $\pm$ 0.12	0.29 $\pm$ 0.12
T <sub>½</sub>	H	64.1	65.6	64.9
T <sub>max</sub>	H	24.6	24.8	24.7
C <sub>max</sub>	ng/mL	4.3	6.6	8.9
Dn-C <sub>max</sub>	ng/mL /µg	0.0355	0.0367	0.0369
AUC <sub>0-∞</sub>	ng.h/mL	511.7	815.2	1080.0
Dn-AUC <sub>0-</sub>	ng.h/mL /µg	4.3	4.5	4.5

Dn = Dose-normalized.



subjects because of a too-low response (240 µg FSH-CTP and 150 IU rFSH, respectively). Three subjects who received hCG did not continue with oocyte pick-up because of absence of sperm (120-µg group) or because of too few preovulatory follicles (120 and 180 µg, respectively). In total, six subjects in the FSH-CTP dose groups who had oocyte retrieval did not proceed with ET because of fertilization failure or the recovery of no or too few embryos.

### ***Stimulation characteristics***

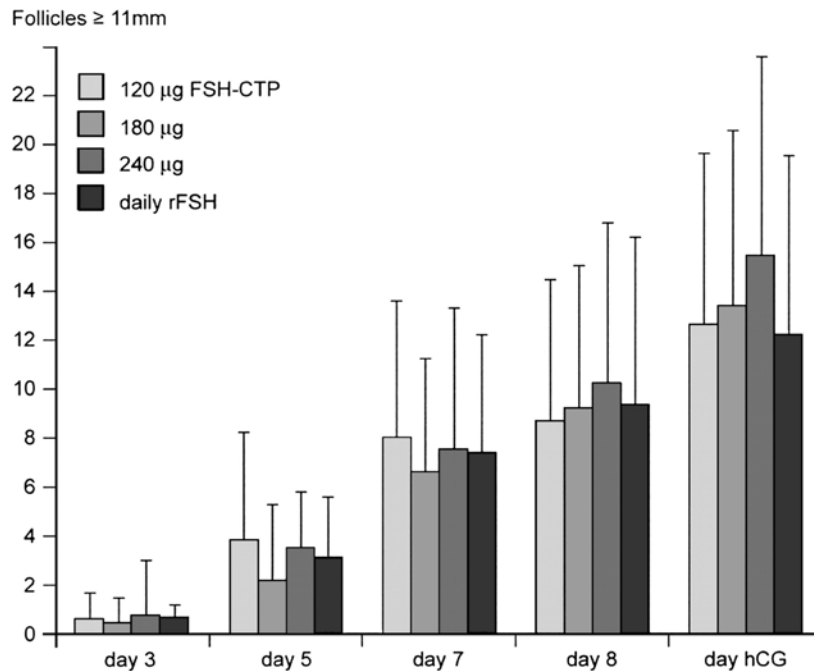
There were no statistical differences between the four treatment groups with respect to the duration of stimulation or the total amount of rFSH administered from d 8 onwards. The median duration of stimulation was 10.0 d in each FSH-CTP dose group and 9.0 d in the rFSH reference group. Thus, after a single injection of 120, 180, or 240 µg FSH-CTP, an additional 3 d of treatment with rFSH (on average, 450 IU in each dose group) were needed to reach the criteria for hCG administration. The total amount of FSH needed in the reference group was 1350 (1200–1950) IU. The median starting day of ganirelix was on stimulation d 7.0 in all four treatment arms.

### ***Pharmacokinetic evaluation***

Serum samples were analyzed both in the FSH-CTP immunoassay and in the FSH Delfia. Figure 1 displays a correlation between the FSH-CTP concentrations (after logarithmic transformation) measured by means of both assays. A linear model was applied to quantify this relationship and yielded a coefficient of correlation of 0.98. Mean serum FSH-CTP concentrations and derived pharmacokinetic parameters are given in Table 2. For all three FSH-CTP doses tested, the mean elimination half-life ( $t_{1/2}$ ) was approximately 65 h. Furthermore, the dose-normalized AUC and  $C_{\max}$  were similar across doses, implying that the serum concentrations of FSH-CTP were proportional to the dose within the dose range tested. Total serum clearance was 0.237 liters/h and the volume of distribution was 22.1 liters at the mean weight in the population (62.2 kg).

### ***Follicular dynamics***

There were no large differences between the four treatment groups with respect to the total number of follicles of at least 11 mm that developed from d 1 up to the day of hCG (Fig. 2 and Table 3). The initial follicular response (Fig. 2) was highest in the 120-µg group, although on d 5, the numbers of follicles at least 11 mm, at least 15 mm, or at least 17 mm were not significantly different among the four treatment groups. On d 8, subjects treated with 120 µg who received hCG had fewer follicles of at least 15 mm ( $P = 0.05$ ) and fewer follicles of at least 17 mm ( $P = 0.02$ ) compared with those treated with rFSH. In the 180-µg group, only the number of follicles at least 17 mm were significantly lower ( $P = 0.04$ ),



**Figure 2.** The total number of follicles at least 11 mm measured on the various days of stimulation restricted to subjects with hCG administration

whereas on the day of hCG, no significant differences were noted between the numbers of follicles of different size classes.

By means of a linear regression model, a significant dose-response relationship was revealed only for the number of follicles at least 15 mm comparing the three FSH-CTP dose groups ( $P = 0.03$ , data not shown).

#### ***Hormones during the follicular and luteal phase***

Serum concentrations of FSH, LH,  $E_2$ , inhibin-B, and P measured at regular intervals during the follicular and luteal phase up to 2 wk after ET are presented in Fig. 3. During the first days after FSH-CTP administration, serum FSH immunoreactivity measured by Delfia increased with the dose given (Fig. 3A). Thereafter, this immunoreactivity declined to

median values of 8.1, 11.1, and 16.1 IU/liter on d 8 and 9.0, 11.2, and 13.9 IU/liter on the day of hCG in the 120-, 180-, and 240- $\mu$ g groups, respectively. Because of daily administration of rFSH, serum FSH increased from 6.6 IU/liter (predose d 1) to 8.2 IU/liter on d 8 and to 8.8 IU/liter on the day of hCG. On the day of ET and 2 wk after ET, serum FSH levels were similar in all treatment FSH-CTP groups. During the first days of stimulation, serum LH levels declined in all four treatment groups (Fig. 3B). Median serum LH values increased from d 5 onwards in all treatment groups, except for subjects treated with 240  $\mu$ g who showed increasing LH levels from d 3 onwards. Because of initiation of ganirelix treatment (on average on d 7 in each treatment group), serum LH declined again in all treatment groups, resulting, on the day of hCG, in serum LH levels of 0.9, 0.9, 1.0, and 1.8 IU/liter in the 120-, 180-, and 240- $\mu$ g FSH-CTP and rFSH group, respectively. There were no differences in the serum LH levels during the luteal phase.

The serum  $E_2$  profile during the follicular and luteal phase was similar for all treatment groups (Fig. 3C). Initial rises of  $E_2$ , reflected by serum values on d 5, were higher in the 120- $\mu$ g group ( $P = 0.06$ ) and 240- $\mu$ g group ( $P = 0.03$ ) compared with those in the rFSH group. Initial rises of serum  $E_2$  levels were lower in the 180- $\mu$ g group, but at d 8 and at the day of hCG administration, no significant differences were found between the four treatment arms.

Inhibin-B levels largely varied between subjects within each treatment group, and no statistical difference was found between the four groups on d 5, d 8, or the day of hCG as shown in Fig. 3D. Median values were clearly highest in subjects treated with 120  $\mu$ g, who showed a small temporary decrease of inhibin-B between d 7 and 8.

At all time points, except for the end of the luteal phase, serum P levels were similar between the treatment groups (Fig. 3E). Serum P levels of subjects treated with 120  $\mu$ g FSH-CTP and rFSH had returned to normal early follicular phase values, whereas subjects treated with 180 and 240  $\mu$ g FSH-CTP showed P values that were still raised in an apparent dose-dependent manner. However, these differences did not reach statistical significance in the 180- $\mu$ g group ( $P = 0.08$ ) and the 240- $\mu$ g group ( $P = 0.07$ ) in comparison with rFSH.

**Table 3.** Mean ( $\pm$  SD) number of follicles of different size classes at day 8 and at the day of hCG, restricted to subjects with hCG administration. Treatment group comparison was performed using ANOVA and in case  $P < 0.05$  Dunnett's t-test was performed to compare the 3 FSH-CTP groups.

		Treatment groups				ANOVA P-value
		120 $\mu$ g	180 $\mu$ g	240 $\mu$ g	RFSH	
Day 8						
	$\geq 11$ mm	8.8 $\pm$ 5.6	9.3 $\pm$ 5.7	10.3 $\pm$ 6.6	9.4 $\pm$ 6.9	0.87
	$\geq 15$ mm	2.5 $\pm$ 2.0 (p=0.05)	2.5 $\pm$ 2.0 (p=0.06)	3.8 $\pm$ 3.1 (p=0.93)	4.2 $\pm$ 2.4	0.03
	$\geq 17$ mm	0.8 $\pm$ 1.1 (p=0.02)	0.9 $\pm$ 1.1 (p=0.04)	1.4 $\pm$ 1.5 (p=0.51)	1.9 $\pm$ 1.7	0.03
Day of hCG						
	$\geq 11$ mm	12.7 $\pm$ 6.8	13.5 $\pm$ 7.1	15.5 $\pm$ 8.3	12.3 $\pm$ 7.3	0.46
	$\geq 15$ mm	5.9 $\pm$ 2.5	6.6 $\pm$ 3.1	7.8 $\pm$ 3.1	6.3 $\pm$ 2.4	0.10
	$\geq 17$ mm	3.3 $\pm$ 0.9	3.5 $\pm$ 1.2	4.0 $\pm$ 2.0	3.7 $\pm$ 1.3	0.29

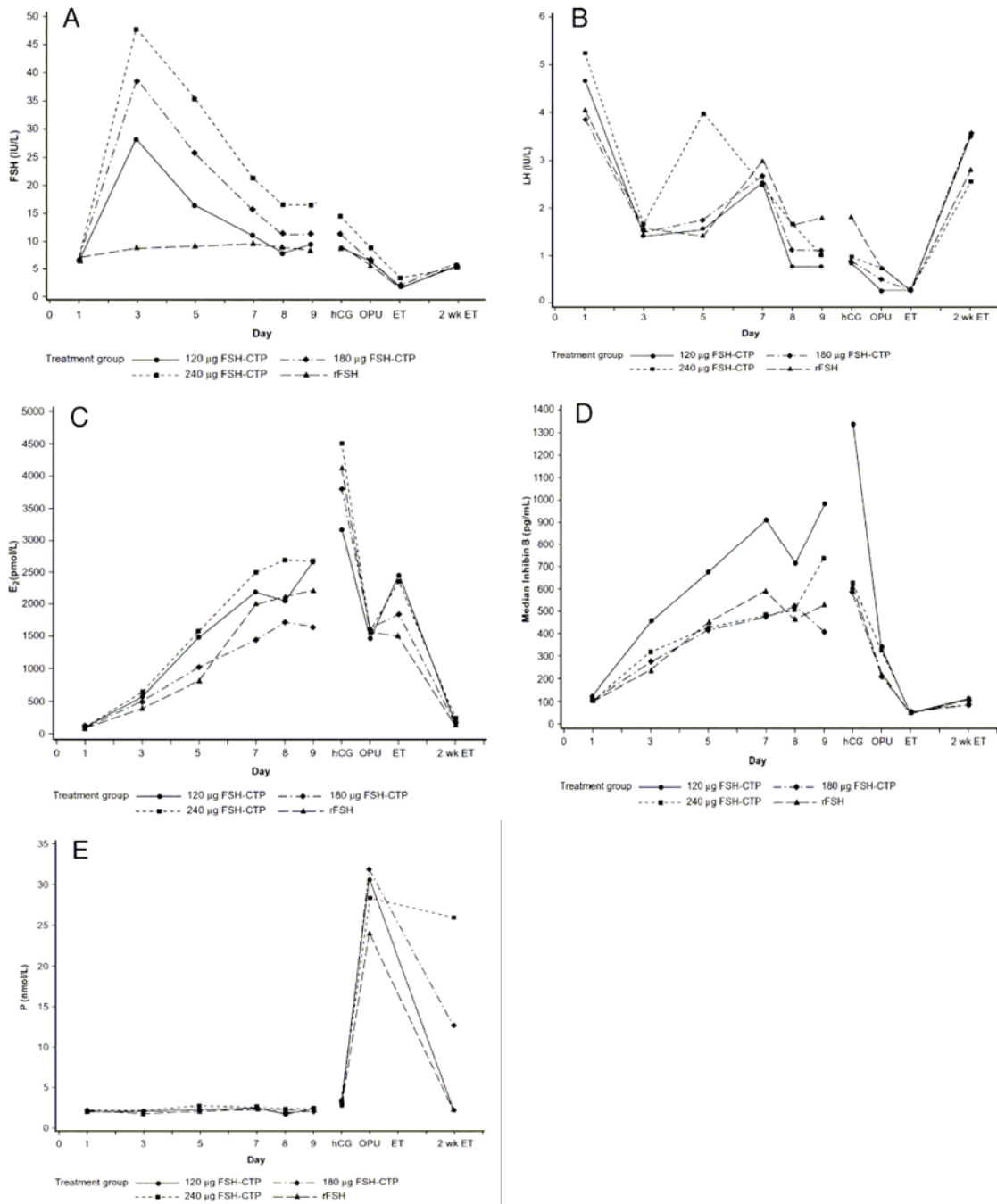
### ***LH rises***

In total, 12 subjects experienced an early LH rise after administration of FSH-CTP on d 1 but before the start of ganirelix treatment: five subjects in the 120-µg group, four subjects in the 180-µg group, and three subjects in the 240-µg group. One subject (240-µg group) had an LH rise during ganirelix treatment. Six of these 13 premature LH rises were accompanied by concomitant P rises ( $>3.2$  nmol/liter). Twelve subjects had oocyte pick-up (mean number of oocytes recovered was 11.1 per attempt), and 10 subjects had ET (mean number of good quality embryos was 3.3 per attempt), of which two subjects became pregnant. Two subjects treated with rFSH experienced a premature LH rise before the start of ganirelix, one subject was discontinued because of a too-low response, and one subject became pregnant. The incidence of premature LH rises was not significantly different between the four groups ( $P = 0.80$  by using the exact  $\chi^2$  test).

### ***Clinical outcome***

The clinical outcome is presented in Table 4. The mean number of recovered oocytes was comparable (mean range, 11.0–12.0) for subjects treated with FSH-CTP and tended to be lower for those women treated with daily rFSH (mean, 7.9). The incidence of metaphase II oocytes recovered in ICSI patients was not different among the groups ( $P = 0.84$ ), i.e. 87, 74, and 74% in the 120-, 180-, and 240-µg dose groups, respectively, and 84% in the daily rFSH group. Whereas the mean number of oocytes recovered per started cycle tended to be higher in three FSH-CTP treatment groups compared with the daily rFSH group, the number of good quality embryos was not statistically different between the treatment groups (range of means, 3.8–4.8 per attempt). Moreover, equal numbers of embryos were available for ET, which was performed on mean (SD) d 3.70 (0.92), 3.57 (0.87), and 3.28 (0.71) in the 120-, 180-, and 240-µg dose groups, respectively, and on d 3.56 (0.84) in the daily rFSH group. In total, 15 ongoing pregnancies were obtained in the FSH-CTP groups and 10 pregnancies in the rFSH reference group ( $P$  value not significant). Ongoing pregnancies included three twins in the FSH-CTP groups and one twin in the reference group. In non-pregnant subjects, menses occurred after hCG administration on d  $16.9 \pm 1.2$  (mean  $\pm$  SD) in the 120-µg group, on d  $17.8 \pm 3.5$  in the 180-µg group, on day  $17.9 \pm 3.8$  in the 240-µg group, and on d  $16.8 \pm 2.0$  in the rFSH group.

## Safety and efficacy of FSH-CTP



**Figure 3.** Median serum hormone concentrations measured during stimulation and during the luteal phase in subjects who received hCG. A, B, C, D, and E represent measured FSH immunoreactivity (IU/liter, Delfia), LH (IU/liter), E<sub>2</sub> (pmol/liter), P (nmol/liter), and inhibin-B (pg/ml), respectively.

**Table 4.** Clinical outcome (mean  $\pm$  SD) of a randomized comparison between three single doses of FSH-CTP and daily rFSH for IVF or ICSI

	FSH-CTP 120 mcg (n=25)	FSH-CTP 180 mcg (n=24)	FSH-CTP 240 mcg (n=25)	rFSH 150 IU/day (n=24)
Number of oocytes per started cycle	11.0 $\pm$ 7.1	11.1 $\pm$ 7.5	12.0 $\pm$ 7.3	7.9 $\pm$ 4.1
Metaphase II oocytes in ICSI	(n=11)	(n=14)	(n=15)	(n=11)
	10.9 $\pm$ 6.9	8.5 $\pm$ 6.3	9.1 $\pm$ 5.5	8.6 $\pm$ 3.0
Fertilization rate	73 $\pm$ 27%	68 $\pm$ 31%	67 $\pm$ 31%	74 $\pm$ 15%
Number of embryos obtained				
Total	8.5 $\pm$ 5.5	6.6 $\pm$ 4.9	7.3 $\pm$ 5.9	5.3 $\pm$ 3.2
Good quality	4.8 $\pm$ 5.0	3.8 $\pm$ 3.3	3.9 $\pm$ 4.1	3.8 $\pm$ 3.4
Transferred	2.0 $\pm$ 0.2	2.0 $\pm$ 0.5	1.9 $\pm$ 0.5	2.0 $\pm$ 0.3
Ongoing pregnancies per started cycle	4/25	5/24	6/25	10/24

### **Safety data**

Ovarian hyperstimulation syndrome (OHSS) occurred in two subjects after treatment with 120 µg FSH-CTP (grade II and III) and in two subjects after treatment with 240 µg FSH-CTP (grade I and II). Two of these four subjects were pregnant. After treatment with daily rFSH, two subjects developed OHSS grade I and II, respectively, and both appeared to be pregnant.

In total, six serious adverse events were reported for five subjects. These reports included bleeding after oocyte retrieval (120 µg), ectopic pregnancy (120 and 180 µg), and three of the six cases of OHSS. In total, eight subjects (32.0%) in the 120-µg group, five subjects (20.8%) in the 180-µg group, eight subjects (32.0%) in the 240-µg group, and four subjects (16.7%) in the rFSH group had at least one adverse event, of which headache was the most frequently reported. No antibodies against FSH-CTP were detected. In general, FSH-CTP was well tolerated in terms of the assessed safety parameters, and no relevant differences between treatment groups were observed.

### **Discussion**

This study is the first to demonstrate, in IVF patients, that a single dose of the long-acting FSH-CTP, a chimeric recombinant glycoprotein hormone, is able to induce multifollicular growth and to maintain the growth of these follicles during an entire week. Although peak serum FSH-CTP levels were reached 2 d after injection and declined thereafter up to d 8 when daily rFSH was started, multiple follicles continued to grow up to a size comparable to those induced by daily rFSH injection. In the current study, all patients started rFSH treatment 1 w after the FSH-CTP injection. Apparently, serum FSH-CTP levels remained above the critical threshold value for substantial follicle development during the first week in all three dose groups, because the cancellation rate before the day of hCG was very low. In addition, the total dose of rFSH required to reach the hCG criteria was similar between the treatment groups, together indicating that the lowest effective dose of FSH-CTP may be lower than the doses tested. Depending on the dose of FSH-CTP and the individual variability in response, some patients may benefit from a lower dose with a shorter time interval up to the start of rFSH, whereas others may be able to reach the criteria for hCG without any additional rFSH.

To date, the bioactivity of circulating FSH-CTP and its threshold to induce multifollicular growth are difficult to explore. Serum FSH-CTP levels are most reliably measured in a specific FSH-CTP immunoassay, which does not cross-react with rFSH. However, future investigators or prescribers of FSH-CTP will not be able to use such a specific assay and may be tempted to measure circulating FSH activity by means of their own FSH EIA. Although this study demonstrates that FSH-CTP cross-reacts in the FSH



EIA (Delfia) in a linear dose-related fashion, its immunoreactivity in this assay cannot be used as an absolute quantitative value because monoclonal antibodies induced against rFSH have a different affinity to FSH-CTP than to rFSH, and the recognition of FSH-CTP is interfered with by endogenous and exogenous FSH. Together this implies that the FSH-CTP (threshold) levels need to be reassessed in clinical experiments and cannot be extrapolated from FSH EIA measurements. In this IVF study, the pharmacokinetics of FSH-CTP appeared to be dose proportional within the dose range of 120–240 µg, and the terminal half-life (~65 h) was dose independent. In a previous FSH-CTP study using pituitary-suppressed female volunteers (Duijkers et al., 2002[▼](#)), the pharmacokinetic properties were almost identical, indicating that these properties are unlikely to be affected by hormonal status or other fertility drugs.

Overall, after a single dose of FSH-CTP, on average only three additional doses of rFSH were needed to reach the hCG criteria, and the total duration of treatment was comparable to daily rFSH treatment. Regardless of the FSH-CTP dose given and whether additional rFSH was needed, the incidence of OHSS was low in this study and did not differ among the treatment groups. Moreover, as in healthy female volunteers (Duijkers et al., 2002[▼](#)), the incidence of side effects in IVF patients was low, and none of the subjects developed antibodies against this chimeric long-acting recombinant FSH agonist.

In this FSH-CTP study, regularly cycling subjects were treated with a GnRH antagonist protocol, which implies that the injected FSH-CTP dose adds up to the natural early follicular FSH rise. In comparison with down-regulated subjects, treatment with daily rFSH in a low-dose GnRH antagonist protocol requires less FSH to reach the same criteria for hCG administration (Borm and Mannaerts 2000[▼](#); The European and Middle East Orgalutran Study Group 2001[▼](#); Fluker et al., 2001[▼](#)). Like rFSH, a certain FSH-CTP dose might be equally effective in a GnRH antagonist and GnRH agonist protocol, although the duration of treatment may become longer after pretreatment with a GnRH agonist. In the current study, applying a flexible initiation of GnRH antagonist administration, subjects in the 240-µg group showed increased median LH levels as early as stimulation d 3 and in all other treatment groups from stimulation d 5 onwards. Comparable rises of endogenous LH have recently been observed in a flexible protocol of GnRH antagonist (at least one follicle of 15 mm) compared with patients who started the GnRH antagonist after 6 d of daily stimulation with rFSH (Kolibianakis et al., 2003[▼](#)). It was reported by the authors that these patients have a significantly lower chance for ongoing pregnancy [8.8% (6 of 68) in the flexible group vs. 23.9% (11 of 46) in the fixed group]. In the current study, patients treated with a flexible protocol and stimulated daily with rFSH showed a similar average increase of LH before the start of the GnRH antagonist treatment. Nevertheless, the implantation rate and pregnancy rate (10 of 24) in this treatment group was extremely high. Using a flexible protocol of GnRH antagonist (started when one follicle reaches a diameter of 14 mm) seems to increase the incidence of premature LH rises (LH > 10 IU/liter) before the start of the antagonist as compared with a fixed GnRH antagonist protocol (started d 6 of stimulation). Premature LH surges were observed on stimulation d 5 or 7 with an overall

incidence of 17.6% in the FSH-CTP-treated subjects and of 8.3% in daily rFSH-treated subjects. Using a fixed protocol, these incidences were 4.3% (Borm and Mannaerts 2000[▼](#)) and 2.7%, respectively (The European and Middle East Orgalutran Study Group 2001[▼](#)), in two studies with a starting dose of 150 IU rFSH and 15% in one study using a starting dose of 225 IU rFSH daily (Fluker et al., 2001[▼](#)). Because approximately 50% of these early rises come along with progesterone rises, implying premature luteinization, a timely start of GnRH antagonist treatment in patients who start in the early follicular phase with a relatively high starting dose of rFSH or FSH-CTP seems to be essential.

To reach the same criteria for hCG administration, subjects treated with FSH-CTP required 1 d more of rFSH treatment compared with subjects treated with a fixed daily dose of rFSH. Although the initial ovarian response to FSH-CTP was appropriate, especially in the lowest-dose group, at d 8, the number of follicles at least 15 mm and at least 17 mm was lower in FSH-CTP-treated subjects. However, the difference was minor and not significant for the 240- $\mu$ g group. In the 120- $\mu$ g group, the growth of follicles tended to progress less between d 7 and 8, when a small decrease of inhibin B was also noted. Previous IVF studies (Casper et al., 2001[▼](#); Fanchin et al., 2003[▼](#)) and the current study have shown that inhibin B levels during daily rFSH administration increase up to d 8 and thereafter plateau. In this study, the decrease of inhibin B levels during stimulation may have been a first indication that serum FSH-CTP levels declined to suboptimal levels to maintain multiple follicular development. On the day of hCG, no differences were noted between the four groups for the number of follicles of the different size classes, indicating that treatment with rFSH reduced the differences noted on d 8 to a large extent in all three FSH-CTP dose groups.

Interestingly, the number of oocytes recovered in all FSH-CTP dose groups was clearly higher compared with the number of oocytes recovered after daily rFSH injection. This observation might be related to the higher total amount of circulating FSH (FSH-CTP and rFSH) from d 8 onwards, maintaining follicular growth up to the day of oocyte retrieval to a larger extent in the FSH-CTP-treated subjects compared with women treated with a fixed dose regimen of 150 IU rFSH. Because there was no statistically significant difference between the four groups with respect to the number of metaphase II oocytes recovered in ICSI patients or the number of good quality embryos, these high FSH levels at the end of the follicular phase may rescue smaller follicles, which finally deliver immature oocytes.

In this study protocol, the flexible time intervals between hCG injection and oocyte pick-up and between oocyte pick-up and transfer, reflecting the clinical practice of the study sites, may have induced additional variability with respect to clinical outcome of this relatively small study. Although not statistically significant, the cancellation rate was higher and the pregnancy rate was lower after treatment with FSH-CTP than after daily rFSH treatment. Whether these observations were made by chance or were related to the stimulation characteristics of FSH-CTP or its regimen remains uncertain until larger comparative trials have been performed.

In conclusion, a single dose of FSH-CTP administered during the early follicular phase of the menstrual cycle appeared to be a potent inducer of multiple follicular growth during a 7-d interval. The total amount of circulating FSH was much higher and declined from d 3 to 8 in subjects who received a single dose of FSH-CTP, but the follicle growth dynamics were very comparable to those induced by daily rFSH administration. Additional studies should establish the optimal FSH-CTP dose and regimen that will provide optimal outcome in different subsets of IVF patients.



## **Chapter 3:**

### **Luteal phase aspects of IVF treatments**

### **3.1 Follicular and luteal phase characteristics following early cessation of gonadotrophin-releasing hormone agonist during ovarian stimulation for in-vitro fertilization**

#### **Introduction**

The use of a gonadotrophin-releasing hormone agonist (GnRHa) to prevent a premature rise in serum luteinizing hormone (LH) concentrations in in-vitro fertilization (IVF) cycles was first described in 1984. Next to exogenous gonadotrophins, GnRHa was applied to induce a reversible hypogonadotrophic state by means of pituitary desensitization (Porter et al., 1984[▼](#)). Consequently, ovarian stimulation with gonadotrophins could be continued for an extended period of time and more oocytes could be obtained. Clinical pregnancy rates per cycle and per embryo transfer were reported to increase with the routine use of GnRHa for IVF (Hughes et al., 1992[▼](#)). However, the use of GnRHa during the follicular phase also impairs corpus luteum function, introducing the need for luteal phase supplementation (Smitz et al., 1987[▼](#)). Defective function of the corpus luteum after cessation of GnRHa may be caused by prolonged blockage of pituitary gonadotrophin release during the luteal phase (Smitz et al., 1988[▼](#)). It was suggested that luteal supplementation would improve endometrial quality and pregnancy rates (Smitz et al., 1988[▼](#)). A meta-analysis comparing pregnancy rates with and without luteal support following ovarian stimulation with gonadotrophins combined with GnRHa suggested indeed that luteal support was beneficial (Soliman et al., 1994[▼](#)).

Several authors have shown extremely low endogenous LH concentrations until 10–14 days after discontinuation of the GnRHa (Donderwinkel et al., 1993[▼](#); Smitz et al., 1992a[▼](#); Sungurtekin and Jansen 1995[▼](#)). It may therefore be postulated that GnRHa could be stopped earlier in the stimulation cycle, allowing pituitary recovery to occur during the luteal phase providing endogenous support of the corpus luteum. Preliminary observations indeed suggest that no premature rises in LH and progesterone concentrations took place in patients in which GnRHa was stopped earlier (Pantos et al., 1994[▼](#)). The objective of the present prospective randomized controlled study was to assess whether early follicular phase cessation of GnRHa still avoids a premature rise in serum LH and to study luteal phase LH and progesterone concentrations without exogenous support of the corpus luteum.

## Materials and Methods

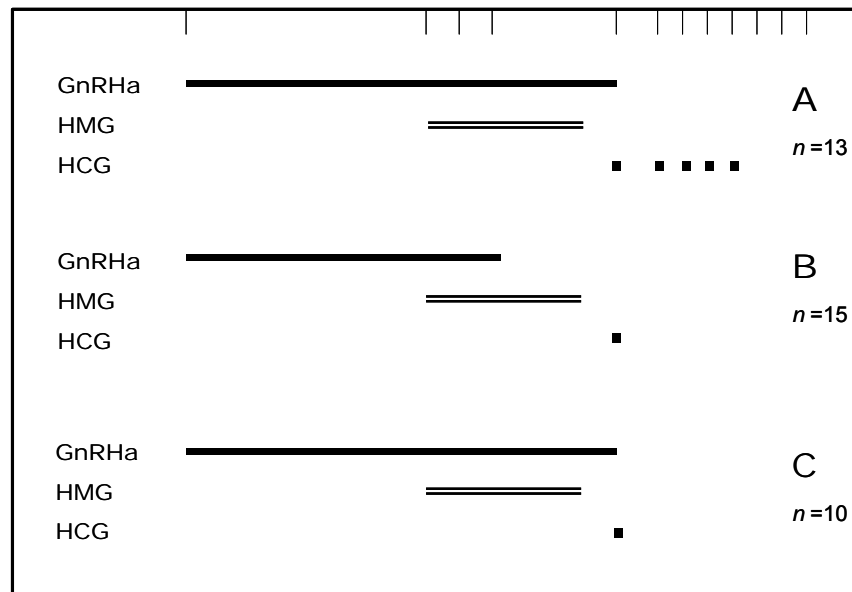
### Patients

The study was approved by the local ethics review committee and a signed informed consent was obtained from all patients. Sixty IVF patients less than 39 years of age were included in the present study. All were having regular menstrual cycles (between 25 and 32 days), and had no known hormonal abnormalities. Indications for IVF included tubal pathology and male factor.

Forty paid volunteers aged 20–34 years with a normal regular menstrual cycle (i.e. 26–30 days), normal body weight (body mass index 19–24 kg/m<sup>2</sup>) and no history of infertility or any endocrine abnormalities served as controls. Daily blood sampling and transvaginal ultrasound was performed, as published previously (Schipper et al., 1998b [v](#)).

### Study protocol

All patients were treated with the so called long protocol. The GnRHa Decapeptyl® (Ferring Nederland B.V., Hoofddorp, The Netherlands) 0.1 mg s.c. daily injections were initiated on cycle day 1. Patients were randomized on the same day (i.e. day 1 of the treatment cycle)



**Figure 1.** Schematic representation of the three different treatment protocols. A routine long gonadotrophin-releasing hormone agonist (GnRHa)/human menopausal gonadotrophin (HMG) protocol with luteal support (group A), early cessation of GnRHa without luteal support (group B), and long use of GnRHa without luteal support (group C). The small vertical lines at the top indicate the days on which blood samples were taken. HCG = human chorionic gonadotrophin

by means of sealed envelopes for one of the three treatment groups A, B or C (20 patients each). Down-regulation was confirmed (reflected by serum oestradiol concentration <150 pmol/l) after 3 weeks GnRHa use, and ovarian stimulation was initiated using human menopausal gonadotrophin (HMG) (Humegon®; N.V. Organon, Oss, The Netherlands) 3 amp/day (=225 IU) i.m. Ultrasound examination was performed from stimulation day 6 onwards every other day until the leading follicle reached a diameter of at least 15 mm. From that day onwards ultrasound examinations were performed daily. Blood samples were drawn on the first day of GnRHa, on the first day of administration of HMG, on the third day of HMG, on each day the patient visited the outpatient clinic for ovarian response monitoring, on the day of human chorionic gonadotrophin (HCG), on the day of oocyte retrieval, and every other day thereafter. Rapid serum oestradiol measurements were performed on day 21 (after 3 weeks use of GnRHa) and following every ultrasound examination. HCG (Pregnyl®; N.V. Organon) 10 000 IU was administered i.m. as a single bolus on the day the diameter of the leading follicle was at least 18 mm and at least 3 follicles >10 mm were present. Oocyte retrieval was performed 35 h later and blood samples were drawn immediately prior to this. Embryos were transferred after 4 days of culture.

Patients in group A received GnRHa until the day of HCG, and subsequent luteal support; HCG 1500 IU i.m. on the day of oocyte retrieval and 2, 4 and 6 days thereafter (conventional long protocol). Patients in group B stopped GnRHa on the third day of HMG stimulation and received no luteal support (see Figure 1). Group C used GnRHa until the day of HCG and received no luteal support. To diminish the risk of ovarian hyperstimulation syndrome (OHSS), only patients with oestradiol concentrations below 8000 pmol/l on the day of HCG received luteal support with HCG in group A. In case oestradiol concentrations were above 8000 pmol/l, micronized progesterone (600 mg/day intravaginally) was given and patients were excluded from further analysis. The oestradiol threshold of 8000 pmol/l was arbitrarily chosen. This was based on our own unpublished observations. It has previously been suggested that when a patient is considered to be at particular risk of developing OHSS, it is recommended that progesterone rather than HCG should be used for luteal support (Akande et al., 1996[▼](#)). However, no absolute threshold for oestradiol could be found to predict an OHSS risk (Mathur et al., 1996[▼](#)).

### ***Hormone assays***

Blood samples were centrifuged at 1000 g for 15 min and serum was frozen and stored at -20°C. Serum was assayed for follicle stimulating hormone (FSH), LH, oestradiol, and progesterone concentrations. In addition, HCG concentrations were assayed during the luteal phase. From each patient, hormone assays were performed in the same run. LH and FSH concentrations were measured by immunofluorometric assay (Amerlite, Orto-Clinical Diagnostic, Amersham, UK) as published previously (Schipper et al., 1998a[▼](#)). Oestradiol and HCG concentrations were measured by Coat-A-Count radioimmunoassay (Diagnostic



**Table 1.** Patients and follicular and luteal phase characteristics in 38 women undergoing in-vitro fertilization (IVF) receiving long GnRHa/HMG stimulation plus luteal support by repeated HCG (group A), early GnRHa cessation without luteal support (group B), and long GnRHa/HMG without luteal support (median and range)

	Group A ( <i>n</i> = 13) <sup>a</sup>	Group B ( <i>n</i> = 15) <sup>a</sup>	Group C ( <i>n</i> = 10) <sup>a</sup>
Age (years)	32 (29–38)	32 (26–36)	33 (28–38)
Duration of HMG stimulation (days)	10 (7–13)	10 (8–22)	9 (7–12)
Follicles <sup>b</sup> ( <i>n</i> )	11 (7–16)	10 (3–17)	11 (4–13)
Oocytes retrieved ( <i>n</i> )	10 (8–19)	13 (3–19)	7 (4–17)
Embryos ( <i>n</i> )	5 (0–11)	2 (0–15)	5 (0–10)
Pregnancies <sup>c</sup>	4 (5)	3 (3)	0 (1)

<sup>a</sup>Number of patients in each group after exclusion due to ovarian hyper-response (oestradiol >8000 pmol/l).

<sup>b</sup>Values reflect number of follicles  $\geq 10$  mm on the day of HCG.

<sup>c</sup>Number of pregnancies (positive pregnancy test) after exclusion (and before).

Products Corp. Los Angeles, CA, USA). Progesterone concentrations were measured by radioimmunoassay kits as described previously (de Jong et al., 1974▴).

Intra- and inter-assay variation was less than 3 and 6% for LH, less than 5 and 7% for FSH, less than 11 and 15% for oestradiol, less than 6 and 7% for HCG, and less than 11 and 12% for progesterone. Lower limit of assay sensitivity was 0.09 IU/l for LH, 0.24 IU/l for FSH and 0.5 nmol/l for progesterone. Cross-reaction of the LH and FSH assay with the HCG injected was <0.1%.

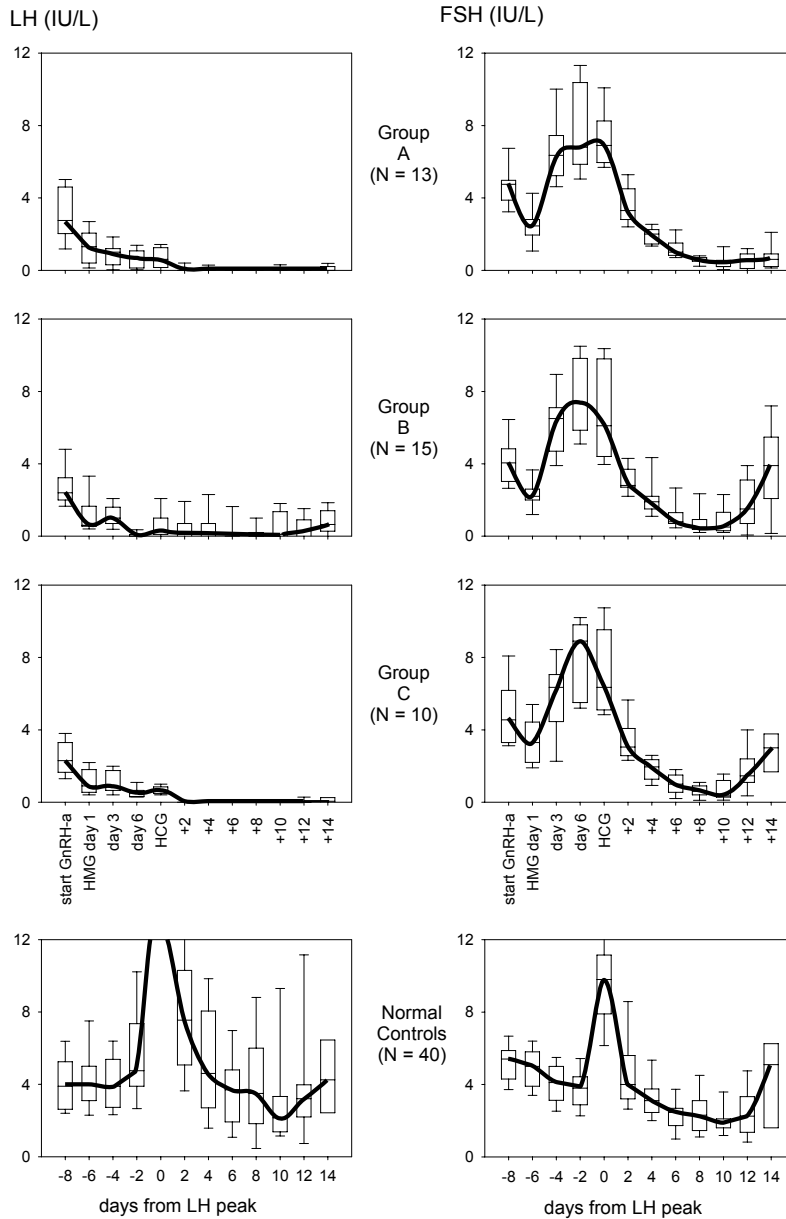
### Statistical analysis

Potential differences in patients' ages and oestradiol concentrations on the day of HCG were tested using the Kruskal–Wallis test. To determine differences in luteal phase hormone profiles the mean area under the curve (AUC) was calculated for LH, FSH, and progesterone during the luteal phase. *P* values below 0.05 were considered to indicate significant differences. Statistical analysis was performed using commercially available software packages (GraphPad prism; Statistical Package for Social Sciences, SPSS, SPSS inc. Chicago, IL, USA).

## Results

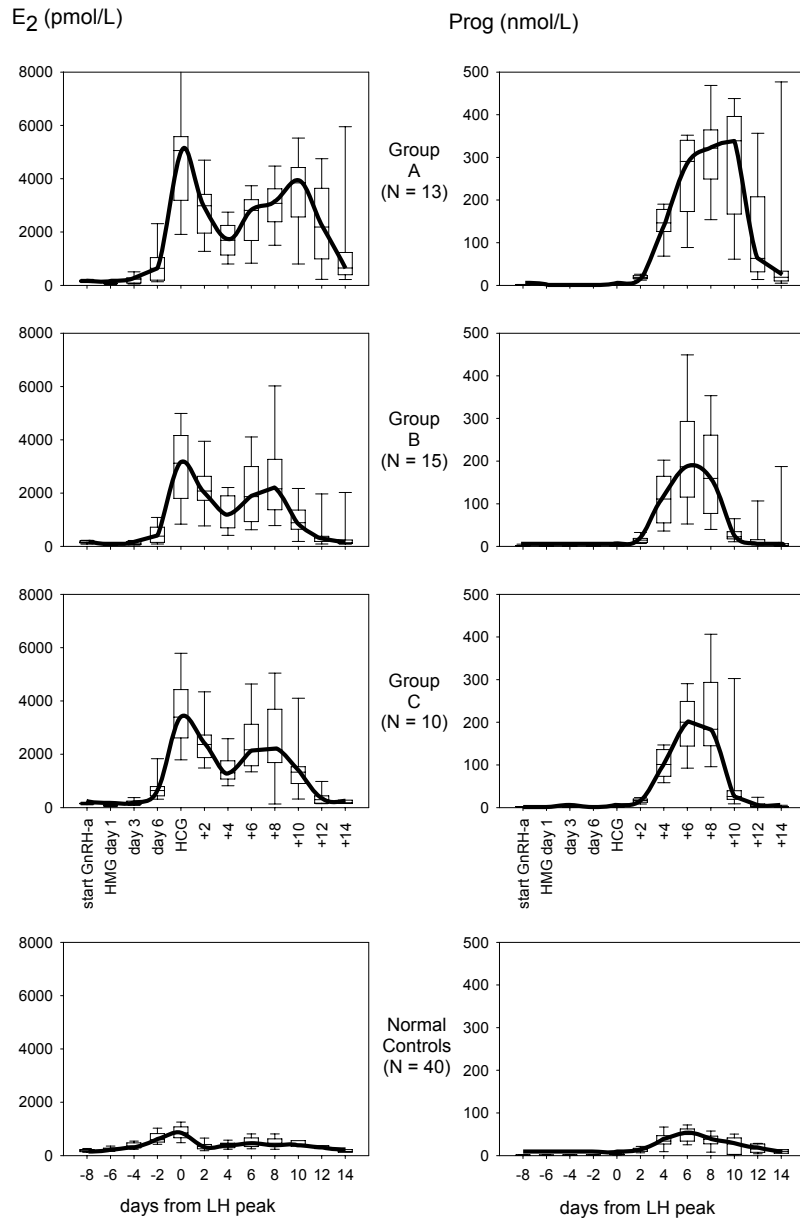
After randomization, 20 patients were included in all three groups. In group A 13 patients were analysed, in group B 15, and in group C 10. The major reason for exclusion was oestradiol concentrations above 8000 pmol/l on the day of HCG ( $n = 5, 3$  and  $7$  for groups A, B and C respectively). Other reasons were treatment cancellation due to poor response ( $n = 1, 1$  and  $0$  for groups A, B and C respectively), a sudden decrease in husband's sperm count after illness ( $n = 1, 0$  and  $1$  for groups A, B and C respectively), and spontaneous ovulation before oocyte retrieval (one patient, group C). In addition, one cycle was cancelled for patient's private reasons (group B), and one patient accidentally injected the HCG bolus 24 h too late (group C). After exclusion no significant differences were found comparing the three groups regarding patients' age, duration of stimulation, number of follicles on the day of HCG, and number of oocytes obtained after retrieval (Table 1). As a fixed dose of HMG (i.e. 225 IU/day) was used, the total amount of HMG used was directly related to the duration of stimulation. As a relatively large number of patients was considered to be a drop-out due to oestradiol concentration higher than 8000 pmol/l, potentially a post-randomization bias could have been introduced. For this reason patients' characteristics were also analysed, including those patients showing ovarian hyper-response. Again, no differences were found regarding patients' age, duration of stimulation, number of follicles on the day of HCG, and number of oocytes obtained after retrieval (data not shown). It was concluded that although the drop-out rate was high, no obvious post-randomization bias was introduced. In group A, five patients became pregnant (defined as a positive urinary pregnancy test 17 days after oocyte retrieval), one of them was not analysed due to an oestradiol concentration above 8000 pmol/l on the day of HCG. In group B, three patients became pregnant of whom all were analysed. In group C, one patient became pregnant (not analysed due to high oestradiol concentrations). In the analysed patients, six pregnancies were ongoing. Ongoing pregnancies ( $n = 6$ ) ended in the birth of four healthy singletons and two healthy twins. The remaining pregnancy was a singleton pregnancy that ended in an early abortion. This patient was in group A. LH and FSH concentrations are depicted in Figure 2. Median LH concentration on the day of HCG were 0.6 IU/l (range <0.09–1.5), 0.4 IU/l (range <0.09–2.5) and 0.7 IU/l (range 0.4–1.0) for groups A, B and C respectively [ $P = 0.37$  not significant (NS)]. In the control group median LH concentration on the day before LH surge was 4.8 IU/l (range 1.3–10.4). No premature LH rises (defined as LH concentrations above 5 IU/l) could be observed in group B patients who stopped GnRHa earlier. The median duration between GnRHa cessation and day of HCG was 7 days (range 4–10).

## The luteal phase in IVF after GnRH agonist



**Figure 2.** Box and whisker plots representing median values and 25th and 75th percentiles of luteinizing hormone (LH) and follicle stimulating hormone (FSH) serum concentrations in three different in-vitro fertilization (IVF) treatment protocols and normal ovulatory controls. Long GnRH $\alpha$ /HMG protocol with luteal support (group A), early cessation of GnRH $\alpha$  without luteal support (group B), and long use of GnRH $\alpha$  without luteal support (group C).

## The luteal phase in IVF after GnRH agonist



**Figure 3.** Box and whisker plots representing median values and 25th and 75th percentiles of oestradiol and progesterone serum concentrations in three different IVF treatment protocols and normal ovulatory controls. Long GnRHa/HMG protocol with luteal support (group A), early cessation of GnRHa without luteal support (group B), and long use of GnRHa without luteal support (group C).

Extremely low LH concentrations were found in the luteal phase in groups A and C (most concentrations below assay sensitivity). The mean area under the curve (AUC) in group B (4.8) was significantly higher compared to groups A or C (0.4, and 0.3 respectively) ( $P = 0.01$  and  $0.02$  respectively) but significantly lower ( $P < 0.001$ ) versus controls (39.5). Median FSH concentrations on the day of HCG were 6.9 IU/l (range 5.6–10.4), 6.0 IU/l (range 3.9–10.2), 7.2 IU/l (range 4.8–10.8), and 3.8 IU/l (range 1.8–6.3) for groups A, B, C, and controls respectively. Compared to the control group, luteal phase FSH concentrations were significantly lower in group A ( $P = 0.001$ ) but similar for groups B and C. In groups B and C (and in controls) a rise in serum FSH concentrations was observed from day 10 following HCG onwards. This rise was absent in group A. The median rise in FSH concentrations between day 10 and 14 was 0.0, 2.6, 2.6 and 0.5 IU/l for groups A, B, C and controls respectively. This was significantly different ( $P = 0.005$ ) between groups.

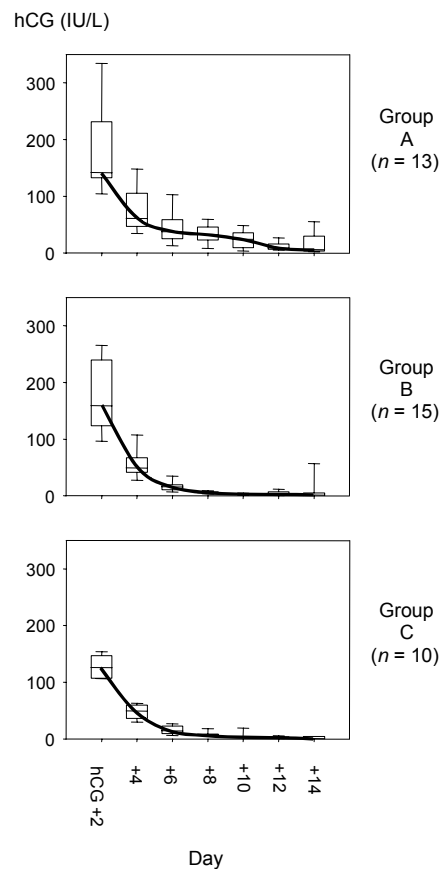
Figure 3 depicts oestradiol and progesterone concentrations in different groups. There was no indication of a premature progesterone rise in the late follicular phase. Luteal phase progesterone concentrations as reflected by the mean AUC were higher in group A (2.4) as compared to groups B and C (1.2 and 1.2 respectively) ( $P < 0.001$ ). There was a significantly higher maximum luteal progesterone concentration in group A [358.6 nmol/l (range 183.6–490.0)] compared to group B [182.3 nmol/l (range 48.2–460.9)] and C [200.3 nmol/l (range 114.0–406.2)] ( $P = 0.02$ ). AUC and maximum concentrations for progesterone were higher in groups B and C versus controls ( $P < 0.001$ ). Moreover, progesterone decrease started significantly later in group A [day 10 (range 8–10 after HCG)] versus B and C [both groups day 8 (range 6–8 after HCG)] ( $P = 0.0005$ ).

Progesterone concentrations and mean AUC were similar for groups B and C. Progesterone serum concentration decreased more gradually in controls (due to reduced maximum concentrations) and this decrease started between days 6 and 8 following the LH surge (i.e. 4–6 days after ovulation). Similarly, luteal phase oestradiol concentrations in group A were significantly higher ( $P < 0.0001$ ) as compared to groups B and C. In turn, oestradiol concentrations in groups B and C were higher as compared to controls ( $P = 0.005$ , and  $P = 0.002$  respectively). Late luteal oestradiol changes were similar to changes in progesterone. Oestradiol serum concentrations also decreased later in group A compared to groups B and C.

HCG concentrations are shown in Figure 4. Mean AUC for groups A, B and C were 554, 347 and 290 respectively, this is significantly different between groups ( $P = 0.005$ ). In group A, HCG was administered during the luteal phase. The relationship between serum HCG and progesterone concentrations during the luteal phase is described in Figure 5, separately for all three groups. In pregnant patients, HCG concentrations started to rise between days 12 and 14 after HCG.

## Discussion

The co-administration of GnRHa in IVF has improved overall treatment outcome (Hughes et al., 1992<sup>▼</sup>). However, with the use of GnRHa the late luteal phase progesterone production was inadequate in women not receiving luteal support (Smith et al., 1989<sup>▼</sup>; Smitz et al., 1988<sup>▼</sup>). GnRHa is routinely continued until oocyte retrieval criteria are met, but pituitary suppression continues after stopping GnRHa (Sungurtekin and Jansen 1995<sup>▼</sup>). Three studies were previously conducted in which GnRHa was stopped earlier (Faber et al., 1998<sup>▼</sup>; Pantos et al., 1994<sup>▼</sup>; Smitz et al., 1992c<sup>▼</sup>). However, luteal support was included in these studies and hormonal measurements were performed on the day of HCG administration only. It was observed that pituitary down-regulation continues following cessation of GnRHa early during ovarian stimulation for IVF. Indeed, pituitary recovery takes an extended period of time, as was also shown following HMG ovulation induction combined with GnRHa in polycystic ovarian syndrome patients (Donderwinkel et al.,



**Figure 4.** Box and whisker plots representing median values and 25th and 75th percentiles of HCG serum concentrations during the luteal phase of three different IVF treatment protocols: long GnRHa/HMG protocol with luteal support (group A), early cessation of GnRHa without luteal support (group B), and long use of GnRHa without luteal support (group C).

1993▾). If impaired luteal progesterone production were caused by prolonged pituitary suppression after GnRHa use, this may normalize if pituitary recovery took place earlier in the luteal phase. In the present prospective, randomized study, it was investigated whether pituitary recovery and endogenous corpus luteum support would occur if GnRHa were stopped early during ovarian stimulation. The entire follicular and luteal phase was studied and normo-ovulatory women served as controls. Follicular-phase characteristics were not different in the three different treatment groups (except for a minor unexplained difference in oestradiol concentrations). Hence, cessation of GnRHa early in the follicular phase did not affect ovarian stimulation by exogenous gonadotrophins. LH or progesterone rises were not observed in any patients prior to HCG. These results confirm previously published observations (Pantos et al., 1994▾). After continuation of GnRHa until HCG, LH concentrations were extremely low during the subsequent luteal phase both with or without luteal support. Several authors also found early and late luteal LH serum concentrations below 1 IU/l after conventional GnRHa use until HCG (Smitz et al., 1988▾; Urbancsek et al., 1990▾; Valbuena et al., 1997▾). LH concentrations remain extremely low for at least 14 days after discontinuation of GnRHa (Donderwinkel et al., 1993▾). This was confirmed in the present study. However, the present study shows for the first time that after stopping GnRHa earlier in the follicular phase, LH concentrations in the late luteal phase partially recover. Smitz *et al.* found an earlier increase in LH concentration in the day of HCG after early cessation of GnRHa but clearly no LH rise or progesterone rise (Smitz et al., 1992c▾). In the present study, from day 8 after HCG, a slight increase in LH concentrations was found, but these levels did not reach concentrations as measured in regularly cycling controls. In the patients who stopped GnRHa on stimulation day 3 the interval between cessation of GnRHa and HCG was 7–13 days. Hence, the observation period from the day of discontinuation of the GnRHa until the 12th day after oocyte retrieval varied from 16 to 22 days in this group. It can be concluded that some pituitary recovery occurs 16–22 days after GnRHa cessation. However, LH concentrations were still below the physiological range (<0.09–1.9 IU/l). Compared to regular cycling controls, in all three study groups more corpora lutea were present to produce steroids in the luteal phase. The higher concentrations of oestradiol and progesterone itself could cause extremely low LH concentrations in the luteal phase by a strong negative feedback mechanism (Gibson et al., 1991▾). FSH concentrations rose in the late luteal phase in both groups without luteal support. This rise may have been secondary to reduced negative feedback associated with decreased progesterone concentrations.

Progesterone production occurred in both groups without luteal support. However, distinctly lower concentrations were reached and an earlier decrease in progesterone production was noted compared to the group with luteal support. This latter observation is in agreement with previous reports (Smitz et al., 1987▾; Valbuena et al., 1997▾). Surprisingly, no difference in progesterone production was found comparing shorter or longer GnRHa use, despite the partial recovery of LH concentrations when GnRHa was stopped earlier. This may be due to a discrepancy between bioactive and

immunoassayable LH following GnRHa use (Meldrum et al., 1984▼), or irreversible luteolysis before the onset of stimulation by endogenous LH. It seems likely that during the early luteal phase the corpus luteum is supported by the HCG bolus (10,000 IU) administered 35 h before oocyte retrieval. This effect lasted for approximately 1 week as can be seen from the relationship between decreasing HCG concentrations and the duration of progesterone production (Figure 5). In patients receiving luteal support by HCG, progesterone concentrations reached a higher maximum as compared to patients without luteal support or normal regularly cycling controls. Furthermore, the increase in progesterone concentrations lasted longer while the decrease started later. This was probably due to higher HCG concentrations in the luteal phase due to HCG supplementation. Progesterone concentrations decreased the moment that HCG levels fell below approximately 30 IU/l in all three groups.

In natural cycles luteal progesterone concentrations increase to a maximum of 70 nmol/l. In all groups, concentrations substantially higher than these were found. The effects of high progesterone concentrations on implantation chances in stimulated cycles are unclear (Pellicer et al., 1996▼). Since pregnancies occurred in all three groups, it seems fair to conclude that the described hormonal features did not preclude implantation. Due to the small sample size, the possibility that implantation chances are impaired cannot be excluded. Withholding luteal support resulted in an earlier decrease in progesterone concentrations. Effects on implantation chances remain unclear. Progesterone concentrations in the control group were at a more steady level. It is unknown whether a sharp decrease in progesterone concentration could be detrimental despite preceding supraphysiological progesterone levels.

In conclusion, this study shows that after cessation of GnRHa earlier in the follicular phase, a premature rise in LH or progesterone concentration is still prevented. In addition, it was found that after earlier cessation of GnRHa, luteal immunoassayable LH concentrations recovered partially. However, no effect on luteal progesterone production could be observed. Progesterone production in IVF patients without luteal support was higher as compared to the natural cycle, but lower compared to patients with luteal support. As progesterone profiles in the luteal phase were not different after earlier discontinuation of GnRHa compared to continuation until HCG, it is concluded that endogenous support of corpus luteum function in the second half of the luteal phase remains insufficient. IVF treatment without luteal support might become a reality when GnRH antagonists become available in the near future. However, other factors potentially involved in impaired luteal phase gonadotrophin secretion such as the preceding bolus injection of HCG or supraphysiological luteal phase steroid feedback should also be considered.

## Acknowledgments



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### **3.2 Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in *in vitro* fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist co-treatment**

#### **Introduction**

The use of GnRH agonist cotreatment in ovarian stimulation protocols for *in vitro* fertilization (IVF) results in a short luteal phase and clearly reduced pregnancy rates (Smitz et al., 1992b[▼](#)), unless luteal phase support is provided (Soliman et al., 1994[▼](#)). In a recent study, the luteal phase in IVF protocols using GnRH agonist without luteal support was characterized by very high progesterone (P) and estradiol (E<sub>2</sub>) concentrations during the early luteal phase (Beckers et al., 2000[▼](#)). Subsequently, premature luteolysis occurred during the midluteal phase. The corpus luteum seemed to be driven by the human (h)CG bolus injection used to induce final oocyte maturation during the late follicular phase, because the decrease in P concentrations was strongly correlated with the decrease in serum hCG levels (Beckers et al., 2000[▼](#)). Endogenous LH levels remained low throughout the luteal phase.

The reason for abnormal luteal function after ovarian stimulation for IVF remains open for speculation. Possible mechanisms involved include: 1) continued down-regulation, attributable to preceding follicular phase GnRH agonist co administration, may retard pituitary recovery (Donderwinkel et al., 1993[▼](#); Smitz et al., 1992a[▼](#)). 2) the induction of multiple follicle development *per se* could either directly or indirectly influence the duration of the luteal phase (Hohmann et al., 2001[▼](#); Messinis and Templeton 1987a[▼](#)). 3) the removal of large quantities of granulosa cells at oocyte retrieval may diminish the most important source of P synthesis by the corpus luteum, thus disrupting the luteal phase (Garcia et al., 1981[▼](#); Vargyas et al., 1986[▼](#)). 4) supraphysiological levels of steroids [related to the higher number of corpora lutea (Smitz et al., 1990[▼](#))] during the early luteal phase could directly inhibit LH release via negative feedback actions at the hypothalamic-pituitary axis (Fauser and Devroey 2003[▼](#)).

The recent availability of GnRH antagonists for the prevention of a premature LH rise in IVF has enabled luteal phase characteristics after ovarian stimulation to be studied in the absence of a GnRH agonist. In contrast to GnRH agonist-induced pituitary desensitization [which suppresses gonadotropin release for at least 2–3 wk after cessation of the GnRH agonist (Beckers et al., 2000[▼](#); Donderwinkel et al., 1993[▼](#); Sungurtekin and Jansen 1995[▼](#)], gonadotropin levels recover within 24 h after stopping the GnRH antagonist (Ditkoff et al., 1991[▼](#); Fattinger et al., 1996[▼](#); Oberye et al., 1999[▼](#)). It has

therefore been widely speculated that luteal phase supplementation may no longer be required in cycles where GnRH antagonist cotreatment is applied (Elter and Nelson 2001[▼](#)). Recent data in intrauterine insemination seem to support this contention (Ragni et al., 2001[▼](#)). However, in IVF cycles in which ovarian stimulation was combined with a GnRH antagonist, the duration of the luteal phase was reduced and LH levels were extremely low (Albano et al., 1999[▼](#); de Jong et al., 2000[▼](#); Tavaniotou et al., 2002[▼](#)). The use of a GnRH antagonist also allows the reassessment of the midcycle hCG bolus on corpus luteum function, because hCG can now be replaced by either endogenous or exogenous LH to induce final oocyte maturation. Several studies have demonstrated the feasibility of inducing an endogenous LH surge by administering a bolus dose of GnRH agonist, both in patients treated with ovarian stimulation alone (Emperaire and Ruffie 1991[▼](#); Gonen et al., 1990[▼](#)) and in patients co treated with a GnRH antagonist (Fauser et al., 2002[▼](#)). Moreover, the recent availability of recombinant (r-)LH enables an exogenous LH surge to be used to induce final oocyte maturation during stimulation (The European Recombinant LH study group 2001[▼](#)). The current study was designed to re-examine luteal characteristics after ovarian hyperstimulation for IVF. The nonsupplemented luteal phase characteristics in patients co treated with GnRH antagonists were studied in women randomized to three different approaches for the induction of final oocyte maturation: r-hCG, r-LH, or an endogenous LH surge induced by a GnRH agonist bolus.

## **Materials and Methods**

### ***Patients***

This prospective randomized two-center trial was approved by the local ethics review committees of both participating centers, and a signed written informed consent was obtained from all patients. Inclusion criteria were: 1) regular indication for IVF or IVF/intracytoplasmic sperm injection (ICSI); 2) no more than 38 yr of age; 3) regular menstrual cycles (cycle length between 24–35 d); 4) both ovaries present; 5) absence of uterine abnormalities that could impair embryo implantation or pregnancy evolution; 6) body mass index, 18–29 kg/m<sup>2</sup>; 7) no history of poor ovarian response (less than three oocytes in a previous IVF cycle); and finally, 8) no history of moderate or severe ovarian stimulation syndrome.

### ***Study protocol***

After a negative pregnancy test (Clearview, hCG II; Unipath Ltd., Bedford, UK), ovarian stimulation was initiated on cycle d 2 or d 3 using a fixed daily dose of r-hFSH (Gonal-F, Serono; 150 IU sc). The GnRH antagonist (Antide, Serono; 1 mg daily sc) was initiated on

the day that the largest follicle was at least 14 mm in diameter (Cahill et al., 2000<sup>▼</sup>) and was continued up to and including the randomization day.

When at least one follicle was at least 18 mm, randomization was carried out, by sealed envelopes, to one of three approaches for triggering final oocyte maturation. For both centers, a separate stratified randomization list was generated by computer. The three arms of this study were: 1) r-hCG (Ovidrel, Serono), 250 µg sc (Chang et al., 2001<sup>▼</sup>; The European Recombinant Human Chorionic Gonadotrophin Study Group 2000<sup>▼</sup>); 2) r-LH (Luveris, Serono), 1 mg sc (The European Recombinant LH study group 2001<sup>▼</sup>); or 3) GnRH agonist (Decapeptyl, Ferring, Hoofddorp, The Netherlands), 0.2 mg sc. Oocyte retrieval was performed 35 h later. Insemination took place either by routine IVF or by ICSI. In case of fertilization, a maximum of two embryos were transferred after 2–5 d of culture, according to local procedures. No luteal support was provided. In the absence of menstrual bleeding, a urine or serum pregnancy test was performed 15–20 d post randomization.

The ovarian response was monitored with transvaginal ultrasound (TVS). TVS was performed at fixed days in the follicular phase, *i.e.* stimulation d 1, d 6, and on the day of randomization. The frequency of additional TVS depended on the diameter of the largest follicle. When the largest follicle was no more than 12 mm, the patient returned 2 d later; whereas when the largest follicle was more than 12 mm, she returned the next day. This approach enabled the initiation of the GnRH antagonist when the largest follicle was at least 14 mm.

Blood sampling was performed on stimulation d 1, on the day the GnRH antagonist was initiated, on the day of randomization, just before the administration of the randomized medication, on the day of the oocyte retrieval, and every other day thereafter.

### ***Hormone assays***

Blood samples were centrifuged, and serum was frozen and stored at –20° C. Serum was assayed for FSH, LH, E<sub>2</sub>, P, and hCG in the same laboratory. From each patient, hormone assays were performed in the same run. All measurements were performed by immunofluorometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). Intra- and interassay variations were, respectively, less than 5% and less than 8% for FSH; less than 3% and less than 6% for LH; less than 6 and less than 16% for P; less than 8% and less than 11% for E<sub>2</sub>, and less than 6% and less than 7% for hCG.

### ***Study design and statistical analysis***

In a previous study of IVF patients treated with ovarian stimulation combined with a GnRH agonist, hCG (10,000 IU) for induction of final oocyte maturation, and no luteal support, we observed a mean maximum P level in the luteal phase of 230 ± 95 (SD) nM (Beckers et al., 2000<sup>▼</sup>). We hypothesized that in the absence of hCG, a difference in maximum P levels of 80 nM could be expected. To test the hypothesis that substitution of hCG by an exogenous

or endogenous LH surge leads to a reduction in maximum P levels of 80 nM with 90% power at a  $P$  value of 0.05 (two-sided, assuming the overall SD = 95 nM), 30 patients were needed for each group, *i.e.* a total of 90 patients.

When 40 patients had been included, the study was canceled prematurely because of observed premature luteal phase bleeding and extremely low pregnancy rates. Preliminary observations highlighted a wide variation in the luteal phase length, both within and between the study groups (Fig. 1). Moreover, the differences in median maximum P levels between groups were much larger than expected (Fig. 1). Therefore, it was decided to analyze the data by comparing the area under the curve (AUC) per day, in addition to maximum P levels. The AUC/d was calculated from d 4 after randomization (to exclude the influence of the LH surge) until onset of menses. The sum of the daily levels measured was divided by the number of days until menses occurred. This results in estimated mean concentrations of the various parameters per day. This method of calculation results automatically in linear interpolation of missing values, unless missing values occur at endpoints of the interval. However, this did not occur: five patients had one missing assay day, and one patient had 3 missing days, all in interior points of the luteal interval.

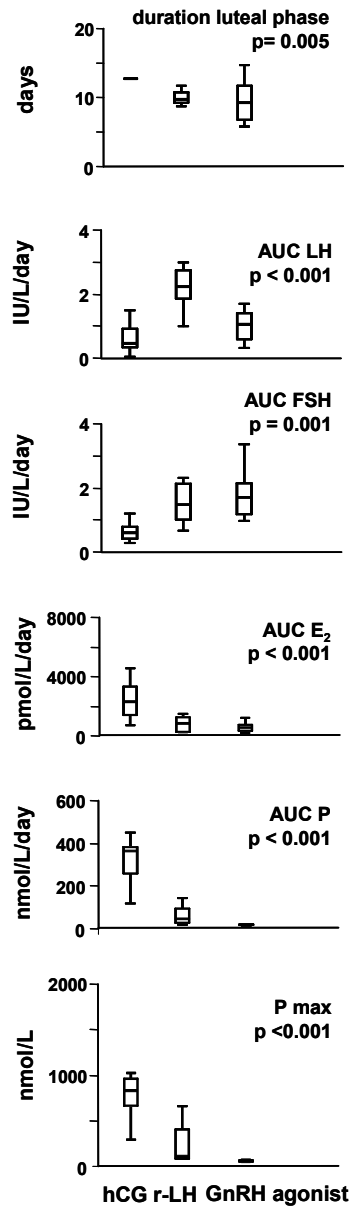
Endocrine profiles of all patients, including the pregnant patients per randomized group, are depicted in Fig. 2. Because blood sampling was carried out until 16 d post randomization, some endocrine data relate to the follicular phase of the next cycle. To address this and to study the endocrine characteristics associated with the length of the luteal phase, we elected to reallocate the non-pregnant patients ( $n = 34$ ) to three groups according to luteal phase duration. Before further analysis, the patients were arbitrarily divided into groups based on luteal phase lengths of no more than 9 d, 10–12 d, and at least 13 d, resulting in 10, 14, and 10 patients, respectively. The Kruskal Wallis test was used to test the different parameters for significance between the three groups. For the number of patients achieving embryo transfer, pregnancy, and ongoing pregnancy, 2-by-3 cross-tables were produced and analyzed by Fisher's exact test. *Post hoc* comparisons between two groups were not performed because no specific hypotheses existed before starting the study. ANOVA on log-transformed data was used to test whether differences among the three randomized groups were dissimilar in the two centers. Associations between continuous parameters were calculated by means of Spearman's rank correlation.  $P$  values  $< 0.05$  were considered to indicate significant differences.

## Results

Before recruitment to the study was completed, it became apparent that the length of the luteal phase was greatly diminished in all three study groups (Fig. 1) and that pregnancy rates were unacceptably low (Table 1). The decision was therefore made to cancel this study after 40 patients were included. At this point, only two pregnancies in the r-hCG and

one pregnancy in the GnRH agonist group had been obtained. This pregnancy rate of 7.5% per started cycle was significantly lower than the 22% previously reported by our group (Hohmann et al., 2003 [▼](#)). One patient, randomized to the r-hCG group, was excluded from further analyses because of premature ovulation.

The overall median age of patients participating in this study was 33.6 (range, 27.4–38.5) yr, median cycle length was 28 (range, 24–32 d) d, and median duration of infertility was 26 (range, 4–105) months. Median early follicular phase FSH levels were 6.5 (range, 2.6–16.3) IU/liter. Median late follicular phase levels (day of randomization) were: E<sub>2</sub>, 4,558 (range, 1,137–34,137) pM; LH, 1.7 (range, 0.5–10.8) IU/liter; and P, 3.3 (range, 1.5–14.6) nM. These pre-randomization parameters did not differ among groups (data not shown).



**Figure 1.** Box (median values and 25th and 75th percentiles) and whisker ( $P_5$  and  $P_{95}$ ) plots representing the differences in the nonsupplemented luteal phase after induction of final oocyte maturation with either r-hCG, r-LH, or GnRH agonist in the duration of the luteal phase (calculated as interval between day of r-hCG, r-LH, or GnRH agonist and onset of menstruation in nonpregnant patients), and AUC/d of LH, FSH, E<sub>2</sub>, and P. To determine differences in luteal hormone profiles, the AUC estimated from d 4 after randomization (to exclude the influence of the LH surge) was divided by the number of days, because the duration of the luteal phase showed a large variation. This value indicates a mean level of LH, E<sub>2</sub>, and P on each day of the luteal phase (in nonpregnant patients). Differences in maximum P (P max) levels among the three groups are shown.

**Table 1.** Follicular and luteal phase characteristics (median and ranges) of 39 subjects undergoing ovarian stimulation for IVF using r-hFSH/GnRH antagonist, randomized for three different strategies for the induction of final oocyte maturation.

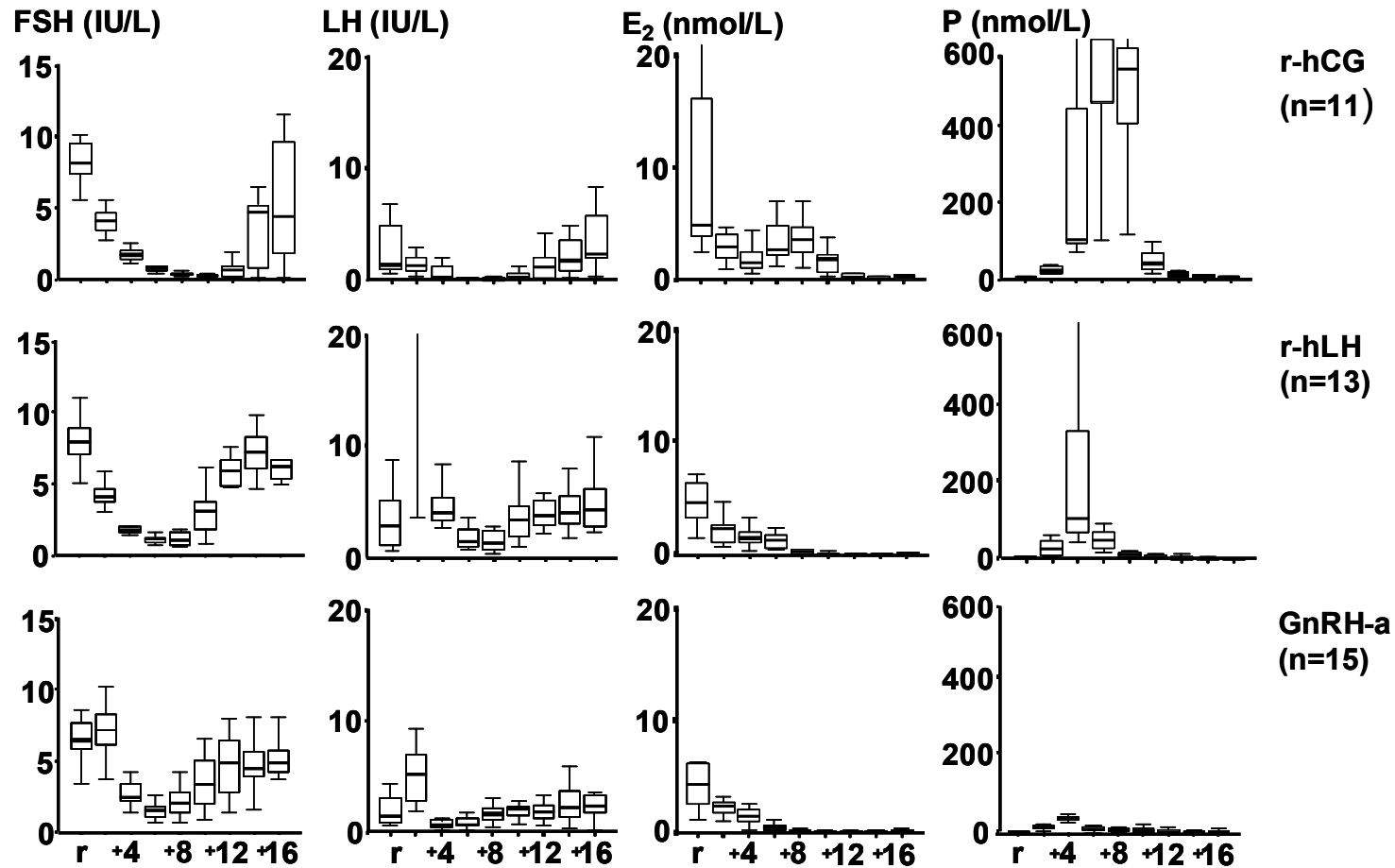
	r-hCG (n=11)	r-LH (n=13)	GnRH agonist (n=15)	P value <sup>1</sup>
Duration follicular phase (d)	11 ( 9 – 14 )	2 (10- 14 )	12 ( 9- 16 )	0.9
No. Days GnRH antagonist	4 ( 3 – 8 )	4 ( 3- 6 )	4 ( 2 – 7 )	1.0
No. Follicles $\geq$ 11 mm	7 ( 5 – 16 )	8 (2 – 18 )	9 ( 3 - 13 )	0.8
No. Oocytes retrieved	7 ( 3 – 23 )	7 ( 1 – 26 )	10 ( 1 – 7 )	0.9
No. patients achieving embryo transfer <sup>2</sup>	9	11	14	0.4
Pregnancy <sup>2</sup>	2 ( 18 % )	1 ( 8 % )	2 ( 13 % )	0.8
Ongoing pregnancy <sup>2</sup>	2 ( 18 % )	0 ( 0 % )	1 ( 7 % )	0.3
LH (day of oocyte retrieval) (IU/L)	1.3 ( 0.3 - 2.9 )	50.6 ( 3.7- 54.1 )	5.5 ( 2.0 - 9.6 )	< 0.001
Day of P <sub>maximum</sub>	6 ( 6 – 8 )	4 ( 4 – 6 )	4 ( 4 – 6 )	< 0.001
Day of decrease of P	8 ( 6 – 8 )	4 ( 4 – 8 )	4 ( 4 – 8 )	< 0.001

<sup>1</sup> Parameters were tested for significance using Kruskal Wallis test.

<sup>2</sup> Calculated per randomized group and tested for significance using a two-tailed Fisher's exact test.



# The luteal phase in IVF after GnRH antagonist



**Figure 2.** Box (median values and 25th and 75th percentiles) and whisker (P<sub>5</sub> and P<sub>95</sub>) plots representing FSH, LH, E<sub>2</sub>, and P serum concentrations in all 39 subjects (with or without pregnancy) in the nonsupplemented luteal phase after induction of final oocyte maturation with either r-hCG, r-LH, or GnRH agonist. On the x-axis, the days of blood sampling are given. r, Day of randomization; +4, 4 d after randomization, *i.e.* day of oocyte pick up; +8, 8 d after randomization; and likewise for +12 and +16.

Follicular phase and luteal phase characteristics of the treatment cycle, comparing the three different oocyte maturation strategies, are shown in Table 1 and Fig. 1. Correcting for center did not change any of these findings. The group by center interaction in ANOVA (which tests for a center effect) was never significant except for P AUC ( $P = 0.04$ ). Prerandomization parameters, including duration of the follicular phase, days of GnRH antagonist administration, and number of follicles of at least 11 mm were not different among groups (Table 1). Clinical outcome parameters, including number of oocytes retrieved, number of embryo transfers, and pregnancy rates, were not significantly different among groups (Table 1). The percentage of mature metaphase II (M II) oocytes could only be assessed in the patients undergoing ICSI. In the r-hCG group, eight patients underwent ICSI, and 85% of the oocytes were M II. For the r-LH group, five patients underwent ICSI, and 80% of the oocytes were M II. Finally, in the GnRH agonist group, eight patients underwent ICSI, and 83% of the oocytes were M II ( $P = 0.9$ ). Hence, it seems that all three methods resulted in adequate final oocyte maturation.

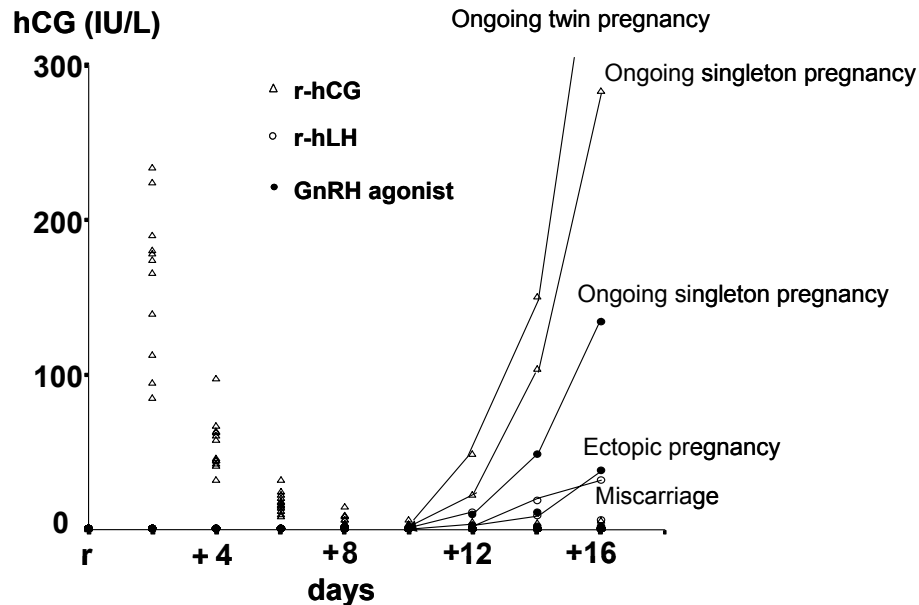
Endocrine profiles of all patients (including the pregnant patients) per randomized group are depicted in Fig. 2. Luteal phase patterns of both gonadotropin and steroid levels were significantly different among groups, as reflected by large differences in the AUC/d for FSH, LH,  $E_2$ , and P (Fig. 1).

The luteal phase hCG levels are depicted in Fig. 3. In patients who received r-hCG to trigger final oocyte maturation, hCG levels remained detectable until 10 d post randomization, as shown before (Beckers et al., 2000 [▼](#)). In pregnant patients, hCG levels began to increase 12 d post randomization, corresponding, on average, with 4 d after implantation. The two pregnant patients treated with r-hCG showed a decline of hCG to 6 and 3 IU/liter on d 10 post randomization. In five patients, a pregnancy was confirmed by a positive pregnancy test between 15–20 d post randomization. Three patients presented with an ongoing pregnancy (*i.e.* two singleton pregnancies and one twin pregnancy). One patient suffered from an ectopic pregnancy and one from an early miscarriage. In five other patients, hCG levels showed a slight rise, up to 6 IU/liter at d 12 to 14 post randomization, despite menses. In these patients, embryo implantation may have occurred without progressing to a clinical pregnancy.

Possible correlations between late follicular phase  $E_2$  concentrations or luteal phase AUC LH, along with correlations between AUC LH and AUC P and duration of the luteal phase for women randomized for either r-LH or GnRH agonist, are shown in Fig. 4.

## Discussion

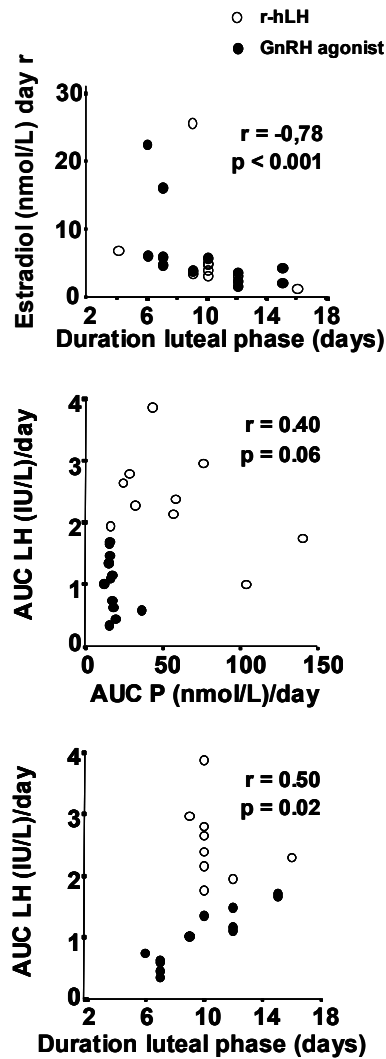
This study demonstrates, for the first time, that the nonsupplemented luteal phase is abnormal after ovarian stimulation and GnRH antagonist cotreatment for IVF. This finding was associated with such a low pregnancy rate that it was deemed unethical to complete



**Figure 3.** hCG levels related to the time after induction of final oocyte maturation with either r-hCG, r-LH, or GnRH agonist. On the x-axis, the days of blood sampling are given.

the study as originally designed. Given that all patients have been co-treated with GnRH antagonists, the degree of abnormality of the luteal phase was striking.

Ovarian stimulation protocols for IVF normally include the co-administration of GnRH agonists to prevent premature luteinization. The resulting down-regulation of the pituitary also leads to highly suppressed luteal phase LH levels, because pituitary recovery after cessation of GnRH agonist takes 2–3 wk (Beckers et al., 2000; Donderwinkel et al., 1993; Sungurtekin and Jansen 1995). In contrast, the recovery of pituitary LH release is almost immediate after the cessation of GnRH antagonist administration (Ditkoff et al., 1991; Elter and Nelson 2001; Fattinger et al., 1996; Obery et al., 1999). This was reported for both Ganirelix and for Cetrorelix available on the market. For Antide, a GnRH antagonist used in the current study, rapid recovery after cessation was also shown after a week of daily injections in males (Fattinger et al., 1996). As-yet-unpublished data also show the clinical feasibility of the use of Antide in IVF patients. A dose-dependent LH suppression was observed, involving daily administration of Antide doses ranging from 0.25–2 mg (Lambalk, personal communication). The results of the current study demonstrate, for the first time, that a rapid recovery of LH release does not occur after ovarian stimulation for IVF and GnRH antagonist cotreatment. Alternative explanations for the abnormal luteal phase under these conditions are therefore warranted.



**Figure 4.** Scatter plots representing the correlation between  $E_2$  levels on the day of randomization ( $r$ ) vs. the duration of the luteal phase (*upper panel*); between the AUC/d of LH vs. the AUC/d of P [both estimated from d 4 after randomization (to exclude the influence of the LH surge) divided by number of days] (*middle panel*); and between AUC/d of LH vs. the duration of the luteal phase (*lower panel*). All three scatterplots represent data after induction of final oocyte maturation with either r-LH or GnRH (not r-hCG).

In the normo-ovulatory cycle, the mid-cycle LH surge induces final oocyte maturation, luteinization of granulosa and theca cells, and rupture of the Graafian follicle (McCracken et al., 1999; Shoham et al., 1995). LH also acts as a luteotropic hormone, because it promotes the growth and the maintenance of the corpus luteum (Filicori et al., 1984; McCracken et al., 1999; Van de Wiele et al., 1970). Indeed, animal and human studies have confirmed that withdrawal of LH (by either cessation of exogenous support in the

hypogonadotropic hypogonadism model or by administering a GnRH analog) induces the initiation of luteolysis (Collins et al., 1986[▼](#); Duffy et al., 1999[▼](#)), although the corpus luteum can survive the lack of support for a limited number of days (Weissman et al., 1996[▼](#)). In stimulated cycles, luteal endocrine characteristics are dramatically altered (Beckers et al., 2000[▼](#); Fauser and Devroey 2003[▼](#); Messinis and Templeton 1987a[▼](#); Smits et al., 1992b[▼](#)), leading to premature luteolysis in non-supplemented patients. As in the natural cycle, the formation and maintenance of corpora lutea in stimulated cycles are also dependent on sufficient support by endogenous LH. When a large bolus dose of hCG is used to induce final oocyte maturation in the late follicular phase, it also acts as a luteotropic agent, and the corpora lutea are supported for 7–10 d (Beckers et al., 2000[▼](#)). After this period, clearance of the exogenous hCG from the circulation is complete, and the maintenance of the non-supported corpora lutea becomes dependent on endogenous LH production. Should LH levels be suppressed in this phase, early luteolysis will occur.

Previously, our group studied LH surge characteristics using different late follicular phase interventions in IVF patients using luteal phase supplementation (Fauser et al., 2002[▼](#)). In the current study, the non-supplemented luteal phase LH profile and corpus luteum function were closely monitored. The LH surge clearly differed between the study groups, with the highest levels assessed on the day of oocyte retrieval occurring in women receiving r-LH. In contrast, LH levels in those women receiving GnRH agonist were low, in agreement with a relatively short duration of the induced endogenous LH surge, as shown previously (Fauser et al., 2002[▼](#)). In both the r-LH group and those receiving GnRH agonist, the duration of the LH surge was relatively short, with median LH levels less than 5 IU/L and less than 2 IU/L, respectively, being observed 4 d post randomization (Fig. 2). Because LH was assessed every other day, the precise characteristics of the LH surges could not be assessed. The differences in observed LH levels did not seem to have any impact on the induction of final oocyte maturation itself, because the percentage of MII oocytes seemed to be normal in all three groups (Junca et al., 1995[▼](#)). However, in those patients receiving r-LH or GnRH agonist, early luteal phase LH levels on d 2 and 4 post randomization were positively correlated to P production, expressed as AUC/d ( $r = 0.62$ ,  $P = 0.002$  for d 2; and  $r = 0.69$ ,  $P < 0.001$  for d 4, respectively). Therefore, the lower the LH levels in the early luteal phase, the lower the P production throughout the luteal phase. This finding is consistent with earlier primate data that showed that LH surges with a duration less than 48 h are insufficient to support, or even induce, the corpus luteum (Chandrasekher et al., 1994[▼](#)).

After the end of the LH surge, continued luteal LH support is necessary to prevent early luteolysis and, subsequently, shortening of the luteal phase. In contrast to the previously described rapid recovery in pituitary function after cessation of GnRH antagonist (Oberye et al., 1999[▼](#)), luteal phase LH levels in all three groups were found to be impaired. Midluteal LH levels represented by the AUC/d for LH (arbitrarily assessed from d 4 post randomization until menses) was also positively correlated with AUC/d for P and with duration of luteal phase (Fig. 4). The longest median duration of the luteal phase was

observed in the r-hCG group ( $P < 0.001$ ), again suggesting extended corpus luteum support by hCG (Fig. 1).

Both luteal  $E_2$  and P concentrations were significantly higher in the r-hCG group, compared with both other groups, which may explain low LH levels through negative steroid feedback activity. The suppressive effect of  $E_2$  on pituitary LH release in the luteal phase has been previously demonstrated (Messinis and Templeton 1987a**[v](#)**). The strong negative correlation in those patients not receiving r-hCG between  $E_2$  levels on the day of randomization (associated with follicle number) and the duration of the luteal phase (Fig. 4) provides further indirect evidence that steroid levels determine luteal phase characteristics. Those patients with a luteal phase length less than 9 d presented with higher median  $E_2$  levels (6238 pM), compared with patients with a longer luteal phase length (3847 and 2263 pM in the groups with a luteal phase length of 10–12 d and  $\geq 13$  d, respectively). These observations imply that mechanisms other than follicular phase GnRH analogue co-treatment are involved in the occurrence of suppressed luteal phase gonadotropins.

In conclusion, our data demonstrate that the luteal phase is insufficient after ovarian stimulation for IVF in combination with daily GnRH antagonist. This is the case whether r-hCG, r-LH, or GnRH agonist is used to trigger final oocyte maturation. The present study suggests that the insufficient luteal phase is principally related to supraphysiological steroid levels in the late follicular and early luteal phase (which are both related to the number of developing follicles and subsequent corpora lutea). Luteal support should therefore be provided after ovarian stimulation combined with GnRH antagonist.

### **3.3 The early luteal phase administration of estrogen and progesterone does not induce premature luteolysis in normo-ovulatory women**

#### **Introduction**

During normo-ovulatory cycles, the corpus luteum remains dependent on support by the pituitary gonadotropins throughout the luteal phase (Filicori et al., 1984[▼](#); Hutchison and Zeleznik 1984[▼](#); Zeleznik and Little-Ihrig 1990[▼](#)). Slowing down of the gonadotropin releasing hormone (GnRH) pulse generator along with diminished luteinising hormone (LH) pulse amplitude, is responsible for the demise of the corpus luteum both in the monkey and the human (Hutchison and Zeleznik 1984[▼](#); Maruncic and Casper 1987[▼](#)). Luteolysis can only be prevented by rising doses of LH (Duffy et al., 1999[▼](#)) or by human chorionic gonadotropin (hCG) (either exogenously administered, or produced by the placenta in case of pregnancy) (Zeleznik 1998[▼](#)). Under normal conditions, a tight balance is operative between negative feedback activity of estradiol ( $E_2$ ), progesterone (P) and the periodic secretion of pituitary LH for corpus luteum support and demise (Gibson et al., 1991[▼](#); Soules et al., 1984[▼](#)). Indeed, under normal conditions luteolysis can be induced by the luteal phase administration of either GnRH agonist (Casper and Yen 1979[▼](#)) or antagonist (Mais et al., 1986[▼](#)).

Since the early days of in vitro fertilization (IVF), it has been described that the luteal phase of stimulated cycles is abnormal. In fact, it was already stated in the first extended report on IVF by Edwards and Steptoe (Edwards et al., 1980[▼](#)) that 'the luteal phase of virtually all patients was shortened considerably after treatment with gonadotropins' and it was suggested that high follicular phase  $E_2$  levels caused by ovarian hyperstimulation might be involved. Initial studies in 1983 also confirmed the occurrence of an abnormal luteal phase in IVF cycles with characteristic features of elevated P levels along with a significantly reduced luteal phase length (Jones 1996[▼](#)).

GnRH agonist co-treatment has been the standard of care in IVF for the prevention of a premature rise in LH during ovarian hyperstimulation (Huirne et al., 2004[▼](#)). Typically, GnRH agonist treatment is initiated in the luteal phase of the preceding cycle and continued until the late follicular phase. Delayed pituitary recovery from down regulation during the luteal phase results in lack of support of the corpus luteum by endogenous LH and therefore in advanced luteolysis (Smitz et al., 1992c[▼](#)). The corpus luteum can be rescued under these circumstances by the administration of hCG (Smith et al., 1989[▼](#)) and this treatment modality became the standard of care for luteal support during the late 80s (Soliman et al., 1994[▼](#)). Because of the association between hCG and ovarian hyperstimulation syndrome (OHSS) (Aboulghar and Mansour 2003[▼](#)), luteal phase hCG

support has been largely replaced by luteal phase P supplementation (Penzias 2002[▼](#); Pritts and Atwood 2002[▼](#)).

Attempts to secure pituitary recovery during the luteal phase by the early follicular phase cessation of GnRH agonist treatment (Beckers et al., 2000[▼](#); Smitz et al., 1992a[▼](#)) failed, presumably due to the prolonged recovery of LH secretion (Donderwinkel et al., 1993[▼](#)). Because of the rapid recovery of pituitary gonadotropin release after discontinuation of GnRH antagonist (Ditkoff et al., 1991[▼](#); Frydman et al., 1991[▼](#)), it has been widely speculated that luteal phase supplementation may not be required following ovarian hyperstimulation in combination with late follicular phase co-administration of GnRH antagonist (Elter and Nelson 2001[▼](#)). Preliminary observations in intra-uterine insemination seem to favor this contention (Ragni et al., 2001[▼](#)). However, various studies in IVF applying GnRH antagonist co-treatment have now clearly established that luteolysis is also initiated prematurely under those conditions resulting in a significant reduction in luteal phase length and greatly compromised chances for pregnancy (Albano et al., 1998[▼](#); Beckers et al., 2003b[▼](#)). More detailed studies could confirm that early- and mid-luteal phase LH levels remained suppressed following the follicular phase administration of GnRH antagonist (Beckers et al., 2003b [▼](#); Tavaniotou et al., 2001[▼](#)).

Alternative mechanisms involved in luteal dysfunction following ovarian hyperstimulation in IVF may be proposed: (A) Follicle puncture and the removal of cumulus-oocyte complexes including large quantities of surrounding granulosa cells (which form the most important P secreting unit in the subsequent corpus luteum). However, initial studies addressing this issue failed to consistently show detrimental effects (Fauser and Devroey 2003[▼](#)). (B) Short loop feedback by the late follicular phase bolus dose of hCG to induce final oocyte meiotic maturation. Although there were some indications of such an effect at the pituitary level suppressing gonadotropin release (Miyake et al., 1976[▼](#)), this could not be confirmed by subsequent studies (Tavaniotou et al., 2001[▼](#); Beckers et al., 2003b[▼](#)). (C) Luteal phase defects can also be induced by stimulating multi-follicle development in normo-ovulatory volunteers (even without follicle aspiration), suggesting a correlation with the development of multiple follicles itself (Hohmann et al., 2001[▼](#)).

Collectively, we postulated that high early luteal phase steroid concentrations could induce advanced luteolysis, due to massive negative feedback resulting in greatly suppressed LH secretion (Tavaniotou et al., 2002[▼](#); Van Der Gaast et al., 2002[▼](#)). The stimulation of multiple dominant follicles during the follicular phase will subsequently give rise to multiple corpora lutea, all involved in luteal phase steroid synthesis. The current study in volunteers was undertaken to further explore the role of early luteal phase steroids in the regulation of pituitary LH release and corpus luteum function. These findings may be relevant for the better understanding of luteal phase dysfunction following ovarian hyperstimulation for IVF.



## Materials & Methods

### *Subjects*

This prospective randomized two-center trial was approved by the local ethics review committees of both participating centers (Erasmus MC Rotterdam the Netherlands (EMC) and AZ-VUB Brussels (VUB)). An information form was sent to responders to advertisements. Subjects interested in participating underwent initial screening before enrollment in the study. A signed written informed consent was obtained from all study participants. Women who completed the study were paid for their participation. Inclusion criteria were: 1) Regular menstrual cycle (cycle length between 24–35 d); 2) Normal body weight (body mass index, 18–28 kg/m<sup>2</sup>; 3) Reproductive age (18–37 yrs); 4) Normal early follicular FSH level ( $\leq 10$  IU/L); 5) Absence of factor V Leiden mutation; 6) No use of oral contraceptive or other hormone related contraceptives in the previous 3 months; 7) No smoking habit; 8) No history of epilepsy, diabetes, gastrointestinal, hepatic, renal or pulmonary disease; 9) No use of other investigational drugs within 3 months prior to the study, or hormonal preparations within one month prior to the study and finally 10) no use of anti depressive drugs.

### *Study protocol*

The initial screening comprised of a medical history, physical examination, including vital signs and vaginal ultrasound examination to exclude abnormalities in both uterus and ovaries. Blood was taken for the assessment of FSH and factor V Leiden mutation (to exclude women with an increased risk for thrombo-embolic processes). After successful screening the volunteers enrolled in the study.

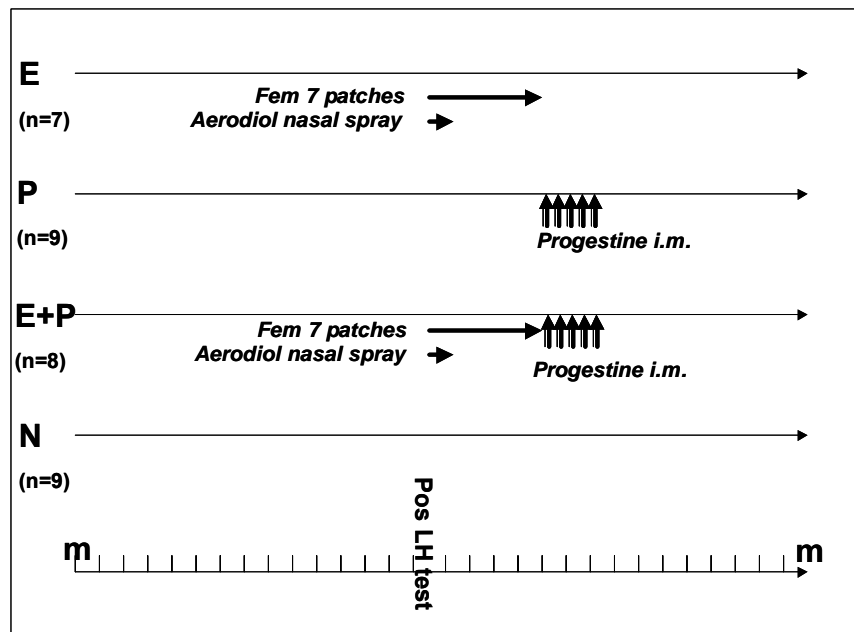
In cycle 1, daily LH tests (Rapi test<sup>®</sup>, Orange medical, Tilburg, the Netherlands) were performed in urine at home starting on cycle day 9 to detect the LH surge (only performed by the 25 women in the EMC). The reason to perform these LH tests was to evaluate the duration of the untreated spontaneous luteal phase. In cycle 2, subjects started the LH tests again on cycle day 9. On the day the LH test became positive (confirmed with a rapid serum LH assay at the VUB), randomization took place for 1 of the 4 treatment groups. In cases where the urinary LH test was unclear an ultrasound scan was performed at the EMC to establish the presence of a pre-ovulatory follicle.

In order to be able to discriminate the effect of the separate steroids as well as the combined effect, this randomized study was designed to include 4 arms: E<sub>2</sub>, P, E<sub>2</sub>+P and no treatment. For both centers, a separate stratified randomization list was generated by computer. Randomization took place by means of sealed envelopes for one of the 4 groups (see also Fig.1). E<sub>2</sub> group: 8 Fem 7 patches (Estradiol 0.1 mg/cm<sup>2</sup> Merck BV, Amsterdam, the Netherlands) applied on the buttocks on the day of the observed LH surge

combined with 4 puffs (600 µg) Aerodiol® (Estrogen 150 µg/spray, Servier, Leiden, the Netherlands) every 3 hours on the day of LH. The patches were removed after the blood sampling on day LH + 4. P group: Prontogest® i.m injections (Progesterone amp, 100 mg/ml, AMSA, Roma, Italia). Started on day LH + 4: evening 25 mg; LH + 5: morning 100 mg, evening 150 mg; LH + 6: morning 300 mg and evening 300 mg. E<sub>2</sub> + P group; Combination of above-mentioned regimens. Non-treatment group: No medication. Blood sampling was performed every other day starting on the day of the positive LH test until LH + 14.

### Justification of interventions

The E<sub>2</sub> intervention was performed earlier in the luteal phase (day LH until day LH + 4) followed by the P intervention (day LH + 4 until day LH + 6). In the E<sub>2</sub> group, we intended to increase the early luteal phase E<sub>2</sub> levels from 800 pmol/l (unstimulated situation (van Santbrink et al., 1995[▼](#))) to 5,000 pmol/L (observed during IVF treatments (Beckers et al., 2003b[▼](#))). In IVF patients, the E<sub>2</sub> levels rise during the follicular phase to maximum levels



**Figure 1.** Schematic representation of the study protocol. Subjects performed urinary LH tests at home. On the day of a positive LH test, randomization took place for either E<sub>2</sub> ((600 µg) Aerodiol® every 3 hours on the day of LH combined with 8 Fem 7 patches from day LH until LH+4); P (Progestine on the day of LH + 4: 25 mg, LH + 5: 100 and 150 mg, LH + 6: 300 and 300 mg, E<sub>2</sub> + P (combination of above mentioned regimens) or controls (non-treatment) group. Blood was sampled every other day during the luteal phase.

on the day of hCG (Beckers et al., 2000▯). From that day onwards, the E<sub>2</sub> serum levels decrease to lower levels at day 4 after hCG. One Fem 7 patch increases E<sub>2</sub> levels to 600 pmol/L (Geyer et al., 1999▯). Therefore, 8 patches were applied on day of the positive LH test, which would give a C<sub>max</sub> of 4800 pmol/l after 16-24 hours. When this C<sub>max</sub> and serial serum levels described earlier in a pharmacokinetic study (Geyer et al., 1999▯) were taken into account, it could be expected to reach the following median E<sub>2</sub> levels: 4800 pmol/L on day LH + 1, 2000 pmo/L on the day LH + 2, 1800 on day LH + 3 and 1500 pmol/L on day LH + 4. As the patches were attached on the day of positive LH test, the E<sub>2</sub> levels on that same day would be slowly increasing (C<sub>max</sub> is only reached after 16-24 hours). In order to reach high E<sub>2</sub> levels on the day of LH, patches were combined with 4 puffs (600 µg) of Aerodiol® nasal spray. One dosis of 300 µg aerodiol increases the E<sub>2</sub> level to 5000 pmol/l after 20 minutes. The initial half-life is 18 minutes, the second half-life is 4 hours (Devissaguet et al., 1999▯). It can be expected to reach levels of around 10,000 pmol/L.

In the P group, we intended to increase P levels from 60 (unstimulated situation) to 800 nmol/L (observed during IVF treatment (Beckers et al., 2003b▯)). P serum levels start to rise at the day 4 after hCG, reaching maximum levels at day 6 and decreasing to low levels at day 10 (Beckers et al., 2000▯; Beckers et al., 2003b▯). The only pharmacokinetic data available in the literature were obtained from oocyte donation patients using P injections 50 mg twice a day for a longer time. The maximum concentration reached in the steady state situation was 120 nmol/l (Devroey et al., 1989▯). These data were used to calculate the dosages needed to reach the desired situation. The expected concentrations with the above-described regimen were 100 nmol/L on day LH + 4, 300 nmol/L on day LH + 5 and 700-800 nmol/L on LH + 6.

### ***Hormone assays***

Blood samples were centrifuged, and serum was frozen and stored at -20 C. Serum was assayed for FSH, LH, E<sub>2</sub>, P and Inhibin A in the same laboratory (EMC). From each patient, hormone assays were performed in the same run. FSH, LH, and P measurements were performed by immunofluorometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). E<sub>2</sub> measurements were done by RIA, Coat-a-count, Diagnostic Products Corp., Los Angeles, CA) and Inhibin A was done by Inhibine A: immuno-enzymometric assay, obtained from Serotec (Oxford, UK). Intra- and interassay coefficients of variation were, < 5% and < 8% for FSH, < 3% and < 6% for LH, < 6% and < 16% for P, < 5% and < 7% for E<sub>2</sub> and < 8% and < 15% for Inhibin A, respectively.

### ***Statistical analysis***

The endpoint of this study was luteal phase characteristics in the four different groups. The particular endpoint used for power calculation was the duration of the luteal phase. The

**Table 1.** Clinical data (median and ranges) of the 33 normo-ovulatory volunteers randomized for either E<sub>2</sub> ((600 µg) Aerodiol® every 3 hours on the day of LH combined with 8 Fem 7 patches from day LH until LH+4), P (Progestine on the day of LH + 4: 25 mg, LH + 5: 100 and 150 mg, LH + 6: 300 and 300 mg, E<sub>2</sub> + P (combination of above mentioned regimens) or no-treatment (controls).

	E <sub>2</sub>	P	E <sub>2</sub> +P	Controls	P value <sup>a</sup>
Duration luteal phase <sup>b</sup> non intervention cycle (days) (n=19)	21 (14-22)	14 (12-15)	14 (12-17)	14 (13-19)	ns
Duration luteal phase <sup>d</sup> intervention cycle (days) (n=33)	15 (13 -16)	14 (11-17)	15 (13-17)	14 (12-17)	ns

<sup>a</sup>ANOVA

<sup>b</sup> Only volunteers in one center (EMC) performed urinary LH tests in the untreated cycle, 3 women were not able to detect the LH surge in the untreated cycle.

three different treatment groups were compared with the non-treatment control. In a previous study from our group involving IVF patients undergoing ovarian hyperstimulation combined with a GnRH antagonist and a GnRH agonist bolus for final oocyte maturation, we observed a mean duration of the luteal phase of 9.6 ( ± 3.3 days ) in the absence of luteal phase supplementation (Beckers et al., 2003b). It was assumed that the mean duration of the luteal phase in the control group would be 14 days. To test the hypothesis whether the exposure of volunteers to high levels of either E<sub>2</sub>, P or both leads to a reduction in luteal phase length with at least 4 days, 80% (β) power at a P value of 0.05 (two sided α 0.025), 9 patients were needed for each group, i.e. a total of 36 patients.

## Results

One hundred and seventy nine women responded to advertisement and received a written information form. After reading this, 56 women decided to participate. The main reason for women deciding not to participate was unwillingness to be treated with high dosages of steroids. Fifty-six women underwent the initial screening and finally 40 enrolled the study. It turned out that the results of the urinary LH tests were not completely reliable. In retrospect (evaluating the LH levels), it became clear that 6 women were randomized (on the day the LH test turned out 'positive') while their LH levels were actually below 10 IU/L. In addition, one volunteer did not reach post-ovulatory P levels in the untreated group. As this protocol was intended as a mechanistic study rather than an intention to treat study,

these women were excluded from further analysis. The pre-randomization characteristics like age, cycle length, body mass index and early follicular FSH were not different between the four groups (data not shown).

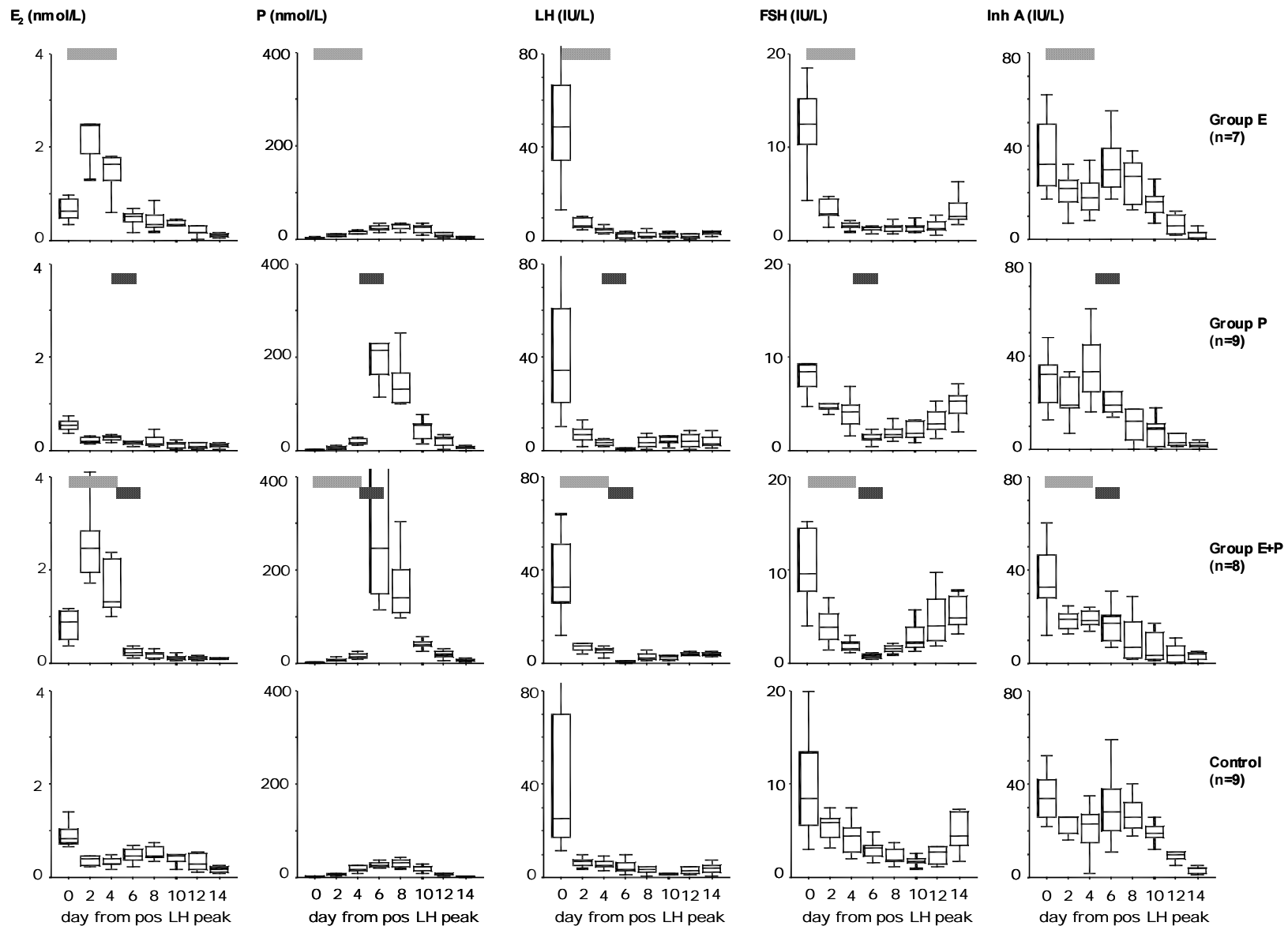
Results on the duration of the luteal phase are shown in Table 1. There was no difference in duration of the luteal phase between the 4 groups. Moreover, in the subjects who also performed the LH tests in the normal cycle preceding the intervention cycle (n=19), there was no difference in duration of the luteal phase between the normal and the study cycles (data not shown).

Endocrine profiles are depicted in Figure 2. The expected median  $E_2$  levels were 4,800 pmol/L on day LH + 1; 2,000 pmol/L on the day LH + 2; 1,800 on day LH + 3 and 1,500 pmol/L on day LH + 4 (see also M & M section). Blood sampling was performed every other day, thus we could only compare the expected and the assessed median levels on day LH + 2 which was 2,470 pmol/L (range 1,310-4,105 pmol/L) and on day LH + 4 which was 1,400 pmol/L (range 596-2,564 pmol/L) in subjects treated with  $E_2$  and  $E_2$  + P. These levels were indeed in the expected range.

After pharmacokinetic calculations regarding P levels, using the little data available (Devroey et al., 1989) it was expected to reach 100 nmol/L on the evening of LH + 4 ( $C_{max}$  was reached after 2 hours), 300 nmol/L on day LH+5, and 700-800 nmol/L on day LH + 6. The assessed median P levels on the day LH + 6 were 216 nmol/L (114-1040 nmol/L) in patients receiving either P or P +  $E_2$ .

The area under the curve (AUC) calculated from the day of a positive LH test until the day LH + 14 (see Table 2) was not different for LH between the 4 groups. However, in order to assess the exact influence of the administered steroids on the LH levels in the mid-luteal phase, the median LH levels were analyzed for each separate day. It was chosen to focus on day LH + 4 until day LH + 10 as the mid luteal phase LH levels were considered the most relevant with respect to the induction of luteolysis. The ANOVA analysis was performed in the total group to test for differences between the 4 groups (n = 33). Furthermore, to be able to differentiate between the separate influence of  $E_2$  and P, the analysis was repeated after rearranging the subjects into two groups twice: supplemented with  $E_2$  (n = 15) or not (n = 18) and supplemented with P (n = 17) or not (n = 16). Results of this analysis are shown in Table 3. LH levels were significantly different between the groups 6 days after the mid-cycle LH surge ( $p < 0.001$ ). This difference was associated with the administration of P ( $p < 0.001$ ), but not  $E_2$  ( $p = 0.16$ ).

## Effect of steroids in the luteal phase



**Figure 2.** Box (median values and 25<sup>th</sup> and 75<sup>th</sup> percentiles) and whisker (P<sub>5</sub> and P<sub>95</sub>) plots representing  $E_2$ , P, FSH, LH and Inhibin A serum concentrations in 33 subjects randomized for the administration of either high dosages of  $E_2$  (▨), P (▩),  $E_2$  + P or no medication (controls) in the early luteal phase. On the x-axis, the days of bloodsampling

**Table 2.** Area under the curves<sup>a</sup> (median and ranges) of E<sub>2</sub>, P, LH, FSH and Inhibin A in 33 volunteers randomized for either E<sub>2</sub> (600 µg Aerodiol® every 3 hours on the day of LH combined with 8 Fem 7 patches from day LH until LH+4), P (Progesterone on the day of LH + 4: 25 mg, LH + 5: 100 and 150 mg, LH + 6: 300 and 300 mg), E<sub>2</sub> + P (combination of above mentioned regimens) or no treatment.

	E <sub>2</sub> (n=7)	P (n=9)	E <sub>2</sub> + P (n=8)	No treatment (n=9)	P value <sup>b</sup>
E <sub>2</sub> (pmol/L)	9,052 (5,902-12,865)	2,221 (1,421-12,865)	6,320 (5,444-10,018)	4,033 (3,039-8,978)	< 0.001
P (nmol/L)	221 (115-276)	961 (641-2,363)	1193 (641-1,668)	252 (157-287)	< 0.001
FSH (IU/L)	21.4 (11.1-37.0)	35.2 (20.2-51.8)	29.2 (24.0-62.8)	42.4 (23.7-57.0)	0.03
LH (IU/L)	41.0 (31.4-65.8)	51.6 (29.5-60.8)	45.5 (26.9-82.6)	47.2 (23.7-144.2)	ns
Inhibin A (IU/L)	218 (117-323)	158 (120-573)	118 (69-252)	250 (155-449)	ns

<sup>a</sup>The Area under the curves were calculated from the day of pos LH surge until day LH + 14

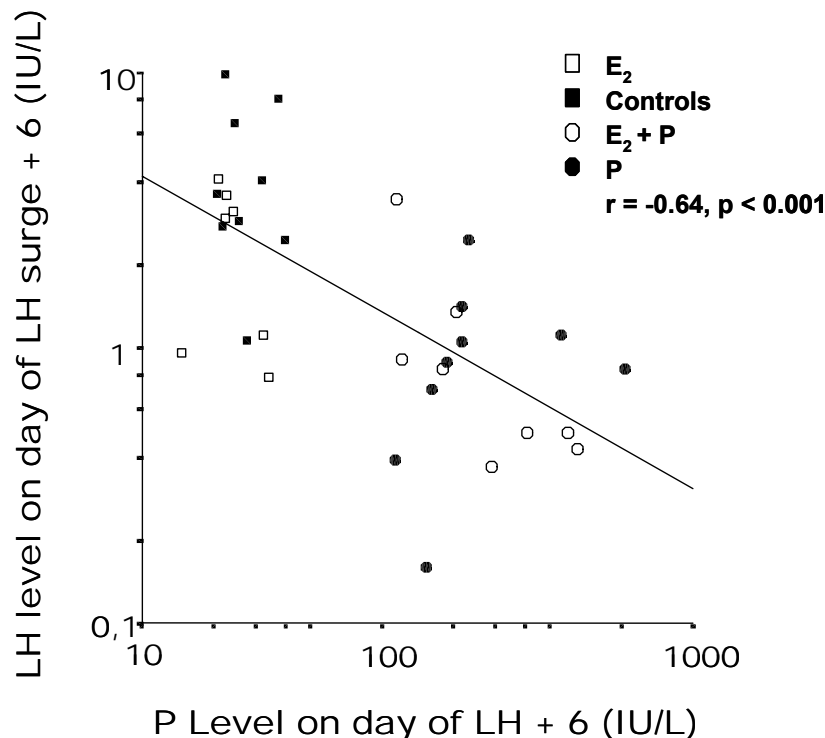
<sup>b</sup>Kruskall Wallis

The AUC calculated from the day of a positive LH test until LH + 14 (see Table 2) was significantly different for FSH comparing the 4 groups ( $p = 0.03$ ). In the  $E_2$  supplemented subjects, the suppression of the FSH levels was more pronounced compared to the P supplemented subjects. As the influence of FSH on the corpus luteum was expected to be low the daily analysis as performed for LH was not done for FSH.

The AUC calculated from the day of the positive LH test until LH + 14 (see Table 2) was not different for Inhibin A comparing the 4 groups. However, the decrease of Inhibin A production, was significantly different in subjects with LH levels  $\leq 1$  IU/L on day LH + 6 ( $n = 13$ ) compared to subjects with LH levels  $>1$  IU/L ( $n = 20$ ) ( $p = 0.001$ ) (see Figure 4)

## Discussion

The current study was designed to assess whether the supraphysiologic steroid levels observed during the early luteal phase after ovarian hyperstimulation for IVF, are to be held responsible for the premature luteolysis and the reduced luteal phase length. The



**Figure 3.** Scatterplot representing the correlation between LH and P serum levels on day 6 after the LH surge in 33 subjects randomized for the administration of either high dosages of  $E_2$ , P,  $E_2 + P$  or no medication (controls) in the early luteal phase.



current study showed that the administration of E<sub>2</sub> or P (or both) in the early luteal phase in normo-ovulatory volunteers did not result in a shortening of the duration of the luteal phase

In the current study we attempted to mimic the endocrine situation of the early luteal phase as found in IVF patients in normo-ovulatory volunteers. The E<sub>2</sub> levels induced by the use of the nasal spray and patches reached the intended values. Unfortunately, P levels found in this study were lower than intended. Either the P doses administered were too low, the duration of the treatment should have been longer or the frequency of the injections should have been higher. However, the P levels were clearly above the physiological range. Maximum P levels were reached on day 6 after the LH surge, which was also the day on which the LH levels were significantly different between the groups. The observed LH levels in the P supplemented groups on day 6 after the LH surge, were significantly lower compared to the LH levels on day 6 after the LH surge in non P supplemented subjects namely 0.67 and 0.89 nmol/L versus 2.97 and 3.64 nmol/L ( $p < 0.001$ ) (see Table 3). A strong correlation was observed between P and LH levels on this day ( $r = -0.64$   $p < 0.001$ ) (See Figure 3). This suggests that the reached maximum P levels were capable of suppressing pituitary LH release. As we were not successful in reaching the intended P levels and maintaining high levels for an extended period of time (as is the case in IVF patients), LH suppression was only achieved for 1 day. This short duration of LH suppression did not induce luteolysis, as we could not find any shortening of the luteal phase. This observation is in line with previous data, suggesting that the corpus luteum can survive without gonadotropin support for up to 3 days (Hutchison and Zeleznik 1985[▼](#)). However, despite the fact that there were no clinical signs of luteolysis, the extremely low LH concentrations on day 6 after the LH surge, resulted in an accelerated decrease to baseline of Inhibin A in subjects with LH levels below 1 IU/L.

Unfortunately it was not possible to determine the endogenous P production by the corpus luteum. First, exogenously administered P was masking P production. Moreover, 17 OHP was assessed as a specific marker for corpus luteum steroid biosynthesis. However, data were also obscured by the conversion of exogenous administered P to 17 OHP. A strong correlation between P and 17 OHP ( $r = 0.68$ ,  $p < 0.001$ ) was observed (data not shown), which may also be due to a cross reaction in the applied assay (de Jong et al., 1984[▼](#)).

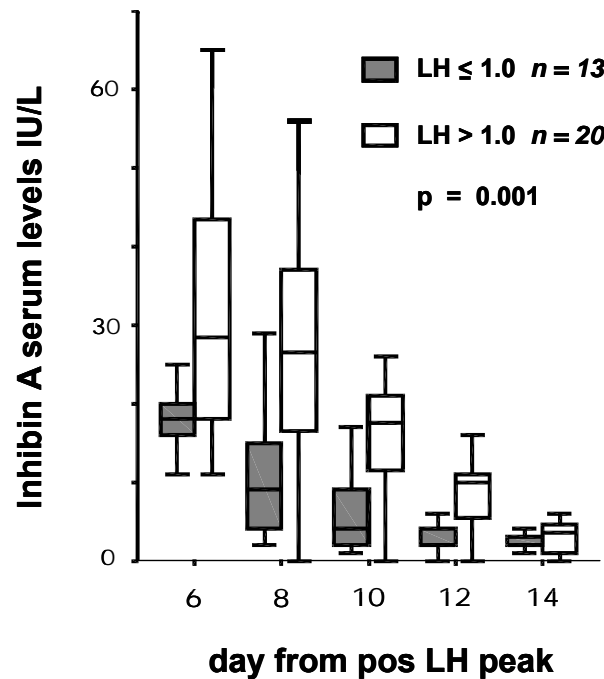
The AUC of FSH was lower in the E<sub>2</sub> supplemented subjects. This suggests a direct negative effect of E<sub>2</sub> at the pituitary gland resulting in a reduced FSH release along with a normal LH output. This in line with earlier data from ovariectomized women (Messinis et al., 2002[▼](#)). As expected, FSH suppression did not result in a reduction of luteal phase length.

## Effect of steroids in the luteal phase

**Table 3:** P values of the ANOVA analysis of the median LH serum levels in the mid luteal phase (day LH + 4, day LH + 6, day LH + 8 and day LH + 10) in 33 volunteers randomized for either E<sub>2</sub> ((600 µg) Aerodiol® every 3 hours on the day of LH combined with 8 Fem 7 patches from day LH until LH+4), P (Progestine on the day of LH + 4: 25 mg, LH + 5: 100 and 150 mg, LH + 6: 300 and 300 mg, E<sub>2</sub> + P (combination of above mentioned regimens) or no treatment.

	Between the 4 groups ( <i>n</i> = 33 )	E <sub>2</sub> supplemented ( <i>n</i> = 15 ) versus non E <sub>2</sub> supplemented ( <i>n</i> = 18 )	<i>P</i> supplemented ( <i>n</i> = 17 ) versus non P supplemented ( <i>n</i> = 16 )
LH + 4	0.26	0.04	0.76
LH + 6	<b>&lt; 0.001</b>	0.16	<b>&lt; 0.001</b>
LH + 8	0.88	0.92	0.68
LH + 10	0.15	0.20	0.11

Previous studies demonstrated that in IVF patients to whom no luteal support was provided, the endogenous P production was much higher compared to normo-ovulatory controls. This has been demonstrated both in the so-called long protocol (Beckers et al., 2000▼) as well as with the use of a GnRH antagonist co-treatment (Beckers et al., 2003▼). In the latter study, P levels were decreasing already from day 6 after hCG, presumably because the corpora lutea were depending on the hCG which had been injected 35 hours before oocyte retrieval and subsequently cleared from the circulation around 8 days after injection. As the exogenous hCG disappeared, luteolysis occurred (and P production stopped) supposedly due to insufficient support by endogenous LH. In support of that hypothesis, we found a positive correlation between the AUC of LH and the AUC of P. Moreover, we previously found a positive correlation between the E<sub>2</sub> levels on the day of hCG and the duration of the luteal phase (Beckers et al., 2003b▼). It has been suggested that in IVF patients, the supraphysiologic levels of E<sub>2</sub> or P produced by multiple corpora lutea in the early luteal phase, results in a profound negative feed-back at the



**Figure 4.** Box (median values and 25<sup>th</sup> and 75<sup>th</sup> percentiles) and whisker (P<sub>5</sub> and P<sub>95</sub>) plots representing Inhibin A serum concentrations in 33 subjects randomized for the administration of either high dosages of E<sub>2</sub>, P, E<sub>2</sub> + P or no medication (controls). The Inhibin A levels were analyzed separately in subjects with an LH level ≤ 1.0 IU/L and > 1.0 IU/L on day 6 after the LH surge. On the x-axis, the days of bloodsampling.

hypothalamic pituitary levels resulting in severely suppressed endogenous LH production.

Large difference still exists comparing the current experimental model in volunteers with IVF patients. It could be speculated that, beside the high P levels in the early and mid luteal phase, additional factors are involved in eliciting advanced luteolysis after ovarian hyperstimulation for IVF. First, in IVF patients, the E<sub>2</sub> levels already increase during the follicular phase. In the current study, it was decided not to start the administration of the E<sub>2</sub> already in the follicular phase of the cycle, as we expected that this would suppress normal development of the follicle and subsequent ovulation. Second, no medication was available to induce sufficiently high and steady E<sub>2</sub> levels immediately after administration. The intended levels could only be reached by the use of multiple patches, which unfortunately would take 12 hours to reach maximum levels (Geyer et al., 1999[▼](#)). To induce high E<sub>2</sub> levels on the day of the LH surge, it was decided to add an E<sub>2</sub> nasal spray. This would give immediate high E<sub>2</sub> serum levels, but followed by a rapid decrease (Devissaguet et al., 1999[▼](#)).

Thirdly, evidence suggests that the ovaries also produce non-steroidal substances other than inhibins capable of blocking the positive-feedback effect of E<sub>2</sub> (Messinis 2003[▼](#)). The production of such a substance, referred to as gonadotropin attenuating factor (GnSAF), takes place in small antral follicles that are present in the ovaries (Messinis 2003[▼](#)). Under normal conditions, GnSAF seems to play a role in the regulation of the sensitivity of the pituitary gland for E<sub>2</sub>. Pituitary sensitivity increases during the late follicular phase as the production of GnSAF decrease (Messinis 2003[▼](#)) resulting in the mid-cycle LH surge. During ovarian hyperstimulation, GnSAF suppresses gonadotropin secretion both in monkeys (Schenken and Hodgen 1983[▼](#)) and in the humans (Messinis and Templeton 1987b[▼](#)). It could be hypothesized that in the luteal phase of IVF patients, high levels of GnSAF are still present resulting in low LH levels and subsequent premature luteolysis.

In conclusion, we were not able to induce a shorter luteal phase in normo- ovulatory subjects by administering high doses of steroids during the early luteal phase. However, a distinct but transient suppression of mid-luteal LH levels could be established. This change resulted in an accelerated decrease of inhibin A indicating a 'partial' luteolysis. It could be hypothesized that we would have shorten the luteal phase length if P levels would have been high for an extended period of time, allowing for extended LH suppression and irreversible loss of corpus luteum function. Alternatively, altered follicular phase events such as increased production of GnSAF may also represent a possible explanation for the observed early luteolysis in IVF patients following ovarian hyperstimulation. Additional studies are required to improve the understanding of mechanisms underlying abnormal corpus luteum function after ovarian hyperstimulation.

## **Chapter 4**

General

Discussion

## **General discussion**

### **Introduction**

In vitro fertilization is a complicated treatment with many side effects, complications and a relatively low efficacy (Macklon and Fauser 2003[▼](#)). Women undergoing ovarian hyperstimulation for IVF are required to take one or two daily subcutaneous injections for several weeks. Their treatment requires frequent hospital visits for ultrasound examination in order to monitor the stimulation and to optimize the timing of the oocyte pick up.

The major risks of IVF are both ovarian hyperstimulation syndrome (OHSS) (Aboulghar and Mansour 2003[▼](#); Delvigne and Rozenberg 2002[▼](#)) and multiple pregnancies (Fauser et al., 2005[▼](#)). While individual chances of OHSS remain difficult to predict, known risk factors include a relatively high response to the ovarian hyperstimulation (Beerendonk et al., 1998[▼](#)), the use of hCG as luteal support (Pritts and Atwood 2002[▼](#)) and pregnancy (Aboulghar and Mansour 2003[▼](#)).

The incidence of multiple pregnancies after IVF is alarming. National IVF registries show that about one third of the IVF pregnancies are twin pregnancies (Fauser et al., 2005[▼](#)). The high incidence of complications in these twin pregnancies have recently lead to elective single embryo transfers in many clinics (Gerris 2005[▼](#)).

Altogether, there is a need for the development of more patient friendly regimens including mild stimulation (aiming at less follicles) and single embryo transfer, without a substantial decrease in pregnancy rates. Many studies have been performed in attempts to improve the pregnancy chances, to reduce the risks of IVF treatments and to improve patients' convenience. The studies presented in the current thesis focus on novel ovarian stimulation regimens aimed at improving patients' convenience and on increasing our understanding of the mechanism underlying the abnormal luteal phase associated with ovarian stimulation.

### **The follicular phase**

In the first part of this thesis, one of the studies performed in the development of a new compound, FSH-CTP is presented. The use of this sustained follicle stimulant (SFS) in ovarian hyperstimulation can significantly reduce the number of injections needed for an IVF treatment, and thus improve patients' convenience. Besides diminishing the number of injections needed for the treatment, by increasing the interval of the injections, the duration of the ovarian hyperstimulation can also be reduced. This can be established by the use of the so-called mild stimulation protocols in which the FSH injections start later in the

follicular phase, on cycle day 5 (Hohmann et al., 2003[▼](#)). With this regimen, only a small number of follicles will develop without reducing the chances of pregnancy. The introduction of GnRH antagonists has opened the way to the development of mild stimulation protocols. Aiming at fewer follicles, this approach promises to improve patients' convenience, reduce the intensity of the side effects and reduce the risk of OHSS (Heijnen et al., 2004[▼](#)). In the dose finding study described in this thesis, there were no large differences between the four used doses of FSH-CTP with respect to the total number of follicles on the day of hCG. In all study groups, more than 10 follicles and 10 oocytes were obtained. In this study the ovarian hyperstimulation was started either on cycle 2 or 3. In reporting the first live birth using FSH CTP, we demonstrated the feasibility of this approach. Future studies might investigate the possibility of inducing the development of fewer follicles by delaying the FSH CTP injection for 2-3 days. Potentially, such an approach might allow for a single FSH injection to cover the entire stimulation phase. Eventually the dose of FSH-CTP could be more individualized according to specific patients' characteristics. Combining different doses and injection interval could be studied in different patient groups.

### **The luteal phase**

Two IVF studies in the second part of this thesis were performed in order to investigate whether it was possible to modify stimulation protocols such that luteal phase support would not be required. Initially, in routine practice, patients were treated with repetitive injections of hCG during the luteal phase. Due to the increased risk of OHSS, most clinics now prescribe micronized progesterone, administered vaginally. Unfortunately, this regimen is associated with increased vaginal discharge. Moreover, these capsules carry the inconvenience of being administered about every eight hours.

With the routine use of GnRH agonists in IVF it became clear that the subsequent luteal phase is insufficient (Penzias 2002[▼](#)). In the unsupported IVF cycles there is an early luteolysis due to low LH levels. Early luteolysis leads to the cessation of P production by the corpora lutea and subsequently, the early onset of menstruation (unless prevented by the administration of either hCG or exogenous P). It is well known that LH levels remain low for a longer period of time after the cessation of the GnRH agonist (Donderwinkel et al., 1993[▼](#)). However, in the first study, stopping GnRH agonist in the early phase of ovarian stimulation did not prevent early luteolysis. Normally it could be expected that endogenous LH production would start again 2 to 3 weeks later (Donderwinkel et al., 1993[▼](#)), i.e. in the mid luteal phase. However, in this study LH levels remained extremely low in patients who stopped the GnRH agonist on stimulation day 3. It can be concluded that administration of GnRH agonists does not represent the only mechanism behind low LH levels and early luteolysis after IVF.

In the second study, again we did not prescribe luteal support. Based on the knowledge that the LH production recovers within 24 hours after cessation of a GnRH antagonist (Oberye et al., 1999<sup>▼</sup>), it was expected that the corpora lutea would be supported by endogenous LH and the luteal phase would be sufficient without luteal support. This would have been a major step towards a more convenient IVF treatment. However, in this study, the duration of the luteal phase was dramatically shortened and the pregnancy rates were extremely low. This was especially the case in patients where the final oocyte maturation was induced by an endogenous (with a GnRH agonist) or exogenous LH surge (with a rLH bolus). The duration of the LH surge was too short to induce luteinization and the endogenous P production was minimal or totally absent. In the patients where the final oocyte maturation was induced by a bolus of rhCG the initial P production was supraphysiological but again early luteolysis was induced. It was concluded that even with the use of a GnRH antagonist luteal phase support could not be omitted.

In the course of performing these studies, it became apparent that the duration of the luteal phase was related to the amount of follicles that had developed. The higher the steroid levels in the late follicular phase and the early luteal phase, the lower the LH levels in the mid luteal phase. This conclusion was the basis for the third study in the second part of this thesis. We intended to test the hypothesis that the supraphysiological levels of steroids in the early luteal phase were the mechanism behind the early luteolysis in IVF patients. Therefore, the endocrine situation in the early luteal of IVF patients was mimicked in volunteers. Unfortunately we were not able to induce early luteolysis in these volunteers.

In this study, we succeeded in reaching the intended early luteal phase levels of  $E_2$ , but the P levels were much lower than intended. P levels only reached high levels on one day in the early to mid luteal phase. These high P levels induced a suppression of LH to extremely low levels without inducing luteolysis. It could be speculated that luteolysis would have occurred indeed if the P levels would have been higher for a longer period of time.

In contrast to the effect of P, the high  $E_2$  levels did not have any effect on the luteal LH levels. Either the supraphysiologic  $E_2$  levels found in IVF patients are not involved in early luteolysis, or the damaging effect takes place in the late follicular phase. If that would be the case, it could be speculated that anti estrogens could play a possible role in preventing early luteolysis in IVF patients in the future.

We can conclude that with the current practice of ovarian hyperstimulation luteal support cannot be omitted. It can be speculated that only with the development of two or three follicles, luteal support would not be needed anymore in IVF treatments. If similar results are to be obtained with respect to pregnancy chances, more research is required to optimize the embryo quality and endometrial receptivity.



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## Summary

## Summary

### Chapter 1

In this chapter the endocrine profiles during the normal menstrual cycle have been described. In addition, a short overview of the history of ovarian hyperstimulation for in vitro fertilization is given. The development of the newly designed long acting FSH preparation has been described. Furthermore, differences between GnRH agonists and GnRH antagonists are discussed as well as the currently used clinical protocols with these GnRH analogues. The impact of the final oocyte maturation performed with hCG has been described and finally the available knowledge on the early luteolysis after ovarian hyperstimulation for IVF has been discussed.

Finally, in this chapter, the objectives of this thesis were described.

### Chapter 2.1

In this chapter the first pregnancy and live birth after ovarian stimulation using a chimeric long-acting human recombinant FSH agonist (recFSH-CTP) for IVF was reported.

A 32-year-old woman with a 7-year history of primary infertility was treated. Ovarian stimulation was done with a single SC injection of 180 µg recFSH-CTP on cycle day 3, followed by daily injections of 150 IU recFSH from cycle day 10 onward, combined with daily GnRH antagonist 0.25 mg SC to prevent a premature LH rise. Final oocyte maturation was induced by 10,000 IU hCG. Twelve oocytes were retrieved. Ten oocytes were fertilized in vitro by intracytoplasmic sperm injection, and from these 10 oocytes, two embryos were subsequently transferred after 3 days of culture. A pregnancy test 2 weeks after ET was positive, and ultrasound investigation revealed an intact, intrauterine, singleton pregnancy after 12 weeks.

In conclusion, the first pregnancy and live birth was achieved after ovarian stimulation using recFSH-CTP for IVF.

### Chapter 2.2

In this chapter, a first feasibility study was described. The efficacy and safety of a single dose of recombinant long-acting FSH (FSH-CTP) were investigated in in vitro fertilization (IVF) patients undergoing controlled ovarian stimulation with a flexible GnRH antagonist protocol.

Eligible subjects were randomized to receive a single dose of 120 µg (n = 25), 180 µg (n = 24), or 240 µg (n = 25) corifollitropin alfa (FSH-CTP) or to start daily fixed doses of 150 IU recombinant FSH (rFSH) (n = 24, reference). Subjects who received a single dose of FSH-CTP continued 1 wk after injection (treatment d 8) with fixed daily doses of 150 IU rFSH (Puregon/Follistim) until the day of triggering final oocyte maturation.

The terminal half-life of FSH-CTP was, on average, 65 h and dose independent. Cycle cancellation before human chorionic gonadotropin (hCG) administration occurred in

only three subjects treated with FSH-CTP. The median duration of stimulation was 10.0 d in each FSH-CTP group and 9.0 d in the daily rFSH group. The total number of follicles at least 11 mm at stimulation d 8 and at the day of hCG administration tended to increase with dose of FSH-CTP, although a significant dose-response relationship was found only for the number of follicles at least 15 mm on the day of hCG ( $P = 0.03$ ). Serum estradiol levels and inhibin-B levels were not significantly different between the four groups on d 8 and on the day of hCG. In total, 12 subjects (17.6%) in the FSH-CTP groups and two subjects (8.3%) in the rFSH group experienced a premature LH rise (defined as  $LH \geq 10$  IU/liter) before the start of the GnRH antagonist ( $P$  value not significant between groups). This relatively high incidence of women demonstrating an early LH rise in the FSH-CTP groups may be related to the higher initial rises of serum estradiol and the use of a flexible GnRH antagonist protocol. The mean number of oocytes recovered per started cycle was higher in FSH-CTP-treated subjects compared with rFSH-treated subjects (significant at  $P = 0.03$  for the 240-  $\mu$ g FSH-CTP group), but no difference could be noted between the number of good quality embryos (range of means, 3.8-4.8 per attempt), and equal numbers of embryos were available for embryo transfer.

In summary, FSH-CTP appeared to be a potent inducer of multiple follicular growth; additional research will be needed to select the optimal FSH-CTP dose and treatment time interval.

### Chapter 3.1

Gonadotrophin-releasing hormone agonists (GnRHa) are widely used in in-vitro fertilization (IVF) for the prevention of a premature rise in luteinizing hormone (LH) concentrations. However, the administration of GnRHa during the follicular phase may also impair subsequent luteal function due to retarded recovery of pituitary gonadotrophin secretion. Therefore, luteal supplementation is generally applied. This chapter contains a study designed to determine whether a premature LH surge would still be prevented after early cessation of GnRHa during ovarian stimulation and whether subsequent luteal phase LH production would be sufficient to support progesterone synthesis by the corpus luteum.

Sixty patients were randomized for three groups: (i) A long GnRHa/human menopausal gonadotrophin (HMG) protocol with luteal support by repeated human chorionic gonadotrophin (HCG) ( $n = 20$ ), (ii) early follicular phase cessation of GnRHa without luteal support ( $n = 20$ ), and (iii) a long GnRHa protocol without luteal support ( $n = 20$ ). Frequent ultrasound and blood sampling was performed during the entire IVF cycle. Forty normo-ovulatory women served as controls.

No premature LH surges were found after early cessation of GnRHa. In this group, some pituitary recovery occurred during the late luteal phase, but this did not affect corpus luteum function. Progesterone concentrations were shown to be dependent on disappearance of the pre-ovulatory bolus of HCG. Pregnancies occurred in all three groups.

In conclusion, early follicular phase cessation of GnRHa is still effective in the prevention of a premature rise in LH. Although some pituitary recovery was observed thereafter, corpus luteum function is still abnormal due to early luteolysis.

### **Chapter 3.2**

Replacing GnRH agonist cotreatment for the prevention of a premature rise in LH during ovarian stimulation for in vitro fertilization (IVF) by the late follicular phase administration of GnRH antagonist may render supplementation of the luteal phase redundant, because of the known rapid recovery of pituitary function after antagonist cessation. The two-center study described in this chapter, was performed to compare nonsupplemented luteal phase characteristics after three different strategies for inducing final oocyte maturation.

Forty patients underwent ovarian stimulation using recombinant (r-)FSH (150 IU/d, fixed) combined with a GnRH antagonist (antide; 1 mg/d) during the late follicular phase. When at least one follicle above 18 mm was observed, patients were randomized to induce oocyte maturation by a single injection of either r-human (h)CG (250 µg) (n = 11), r-LH (1 mg) (n = 13), or GnRH agonist (triptorelin; 0.2 mg) (n = 15). Retrieved oocytes were fertilized by either IVF or intracytoplasmic sperm injection, depending on sperm quality. Embryo transfer was performed 3-4 d after oocyte retrieval. No luteal support was provided. Serum concentrations of FSH, LH, estradiol (E<sub>2</sub>), progesterone (P), and hCG were assessed at fixed intervals during the follicular and luteal phase.

The median duration of the luteal phase was 13, 10, and 9 d for the r-hCG, the r-LH, and the GnRH agonist group, respectively (P = 0.005). The median area under the curve per day (from 4 d post randomization until the onset of menses) for LH was 0.50, 2.34, and 1.07 for the r-hCG, the r-LH, and the GnRH agonist group, respectively (P = 0.001). The median area under the curve per day for P was 269 vs. 41 and 16 for the r-hCG, the r-LH, and the GnRH agonist group, respectively (P < 0.001). Low pregnancy rates (overall, 7.5%; range, 0-18% per started cycle) were observed in all groups.

In conclusion, the nonsupplemented luteal phase was insufficient in all three groups. In the patients receiving r-hCG, the luteal phase was less disturbed, compared with both other groups, presumably because of prolonged clearance of hCG from the circulation and the resulting extended support of the corpus luteum. Despite high P and E<sub>2</sub> concentrations during the early luteal phase in all three groups, luteolysis started prematurely, presumably because of excessive negative steroid feedback resulting in suppressed pituitary LH release. Hence, support of corpus luteum function remains mandatory after ovarian stimulation for IVF with GnRH antagonist cotreatment.

### **Chapter 3.3**

The luteal phase after ovarian hyperstimulation for IVF is insufficient. Therefore, luteal phase supplementation is routinely applied in IVF. Mechanisms behind this premature luteolysis however remain unclear. It may be postulated that this premature luteolysis after ovarian hyperstimulation is due to supraphysiologic steroid levels in the early luteal phase.

In the study described in this chapter, high doses of steroids are administered after the LH surge in normo-ovulatory volunteers in order to investigate whether this intervention gives rise to endocrine changes and a shortening of the luteal phase.

Forty non smoking, normal weight women, between 18-37 yrs of age, with a regular menstrual cycle (24–35 d), received randomly either high dosages of  $E_2$ , P,  $E_2 + P$  or no medication. Blood sampling was performed every other day from the day of the LH surge until LH +14. Duration of the luteal phase and endocrine profiles were main study outcomes.

Early luteal phase steroid concentrations achieved by exogenous administration were comparable with levels observed following ovarian hyperstimulation for IVF. No difference in the luteal phase length was observed comparing all groups. However, a significantly decrease in LH levels could be observed 6 days after the mid-cycle LH surge ( $p < 0.001$ ) in women receiving P, resulting in accelerated decrease of inhibin A production by the corpus luteum ( $p = 0.001$ ).

We concluded that the current intervention of high dose steroid administration shortly after the LH surge failed to induce a shortening of the luteal phase in regularly cycling women. It seems that the induced transient reduction in LH allowed for a timely recovery of corpus luteum function. Other additional factors may be held responsible for the distinct reduction in luteal phase length observed after ovarian hyperstimulation for IVF.

## Chapter 4

This chapter summarizes the conclusions from the studies described in this thesis



## **Samenvatting**

## **Samenvatting**

### **Hoofdstuk 1**

In dit hoofdstuk worden de endocriene veranderingen in de normale menstruele cyclus beschreven. Tevens wordt er een kort overzicht gegeven van de geschiedenis van de ovariële hyperstimulatie voor IVF. De ontwikkeling van een nieuw ontworpen langwerkend FSH preparaat wordt gegeven. Verder worden de verschillen tussen GnRH agonisten en GnRH antagonist bediscussieerd. Daarnaast worden de verschillende klinische protocollen die worden toegepast met zowel de GnRH agonisten als met de GnRH antagonist besproken. De consequentie van de toediening van hCG voor de laatste stappen van de eicelrijping wordt beschreven en tenslotte wordt de beschikbare kennis ten aanzien van de voortijdige luteolyse na ovariële hyperstimulatie voor IVF beschreven.

Tenslotte bevat dit hoofdstuk de doelen van het proefschrift.

### **Hoofdstuk 2.1**

In dit hoofdstuk worden de eerste zwangerschap en de geboorte van een gezonde baby, ontstaan na ovariële hyperstimulatie met een langwerkend humaan recombinant FSH preparaat bij IVF gerapporteerd.

Een 32 jarige patiënte met een onvervulde kinderwens sinds 7 jaar, werd behandeld. De ovariële hyperstimulatie werd verricht met één enkele onderhuidse injectie van 180 µg rec FSH-CTP op cyclus dag 3, gevolgd door dagelijkse injecties van 150 IU rec FSH vanaf cyclus dag 10, gecombineerd met een dagelijkse injectie van een GnRH antagonist 0.25 mg s.c. ter preventie van een voortijdige LH stijging en ovulatie. De laatste fase van de eicelrijping -35 uur voor de eicelpunctie- werd geïnduceerd met een injectie van 10.000 IU hCG. Bij de eicelpunctie werden twaalf eicellen verkregen. Tien eicellen waren rijp, hiermee werd de ICSI procedure uitgevoerd. Twee embryo's werden na 3 dagen kweek in de baarmoederholte geplaatst.

Twee weken na deze embryotransfer bleek de zwangerschapstest positief, en echoscopisch onderzoek –verricht na 12 weken- liet een intacte intra-uteriene eenling zwangerschap zien.

Concluderend werd de eerste zwangerschap en vervolgens geboorte van een gezond kind beschreven na het gebruik van rec FSH-CTP bij IVF.

### **Hoofdstuk 2.2**

In dit hoofdstuk worden de resultaten beschreven van het eerste klinische onderzoek met FSH-CTP. De werkzaamheid en de veiligheid van één enkele dosis van het lang werkend FSH (FSH-CTP) preparaat werd onderzocht in in vitro fertilisatie (IVF) patiënten die ovariële hyperstimulatie ondergingen met het zogenaamde flexibele GnRH antagonist protocol.



De vrouwen die in aanmerking kwamen voor deelname aan het protocol, werden gerandomiseerd voor ofwel een enkele dosis van 120 µg (n = 25), 180 µg (n = 24), of 240 µg (n = 25) corifollitropin alfa (FSH-CTP) ofwel voor het starten met dagelijkse vaste doses van 150 IU recombinant FSH (rFSH) (n = 24, controle groep). Bij de vrouwen die een enkele dosis FSH-CTP kregen, werd de behandeling 1 week na de injectie voortgezet met dagelijkse vaste doses van 150 IU rFSH (Puregon/Follistim) tot de dag van laatste eicelrijping.

De terminale half waarde tijd van FSH-CTP was gemiddeld 65 uur en onafhankelijk van de gegeven dosis. Slechts in drie patiënten die behandeld werden met FSH-CTP, werd de cyclus gestaakt voordat human chorionic gonadotropin (hCG) gegeven kon worden. De mediane stimulatie duur was 10.0 dagen in alle FSH-CTP groepen en 9.0 dagen in de dagelijkse rFSH groep. Het totaal aantal follikels  $\geq 11$  mm. op stimulatie dag 8 en op de dag van hCG leek toe te nemen met de gegeven dosis FSH-CTP. Er was echter alleen een significante dosis respons relatie te vinden voor het aantal follikels  $\geq 15$  mm op de dag van hCG ( $p = 0.03$ ). Serum oestradiol spiegels en inhibin-B spiegels waren niet significant verschillend tussen de 4 groepen op stimulatie dag 8 en ook niet op de dag van hCG. In totaal 12 vrouwen (17.6%) in de FSH-CTP groepen en 2 vrouwen (8.3%) in de rFSH groep hadden een voortijdige LH stijging (gedefinieerd als LH  $\geq 10$  IU/liter) voordat de GnRH antagonist werd gestart (P waarde niet significant tussen de groepen). De verklaring voor deze relatief hoge incidentie van vrouwen met een vroege LH stijging in de FSH-CTP groepen, zou kunnen zijn dat in deze groepen de serum estradiol spiegels eerder stijgen in combinatie met het gebruik van de flexibele start van de GnRH antagonist. Het gemiddelde aantal eicellen -verkregen per gestarte cyclus- was hoger in de groepen vrouwen behandeld met FSH-CTP in vergelijking met de groep vrouwen behandeld met rFSH (significant voor de 240 µg FSH-CTP groep ( $p = 0.03$ )). Echter, er werd geen verschil gevonden in het aantal embryo's van goede kwaliteit (variërend van 3.8-4.8 per poging). Bovendien was er een gelijk aantal embryo's beschikbaar voor de embryotransfer in iedere groep.

Concluderend, blijkt FSH-CTP een geschikt middel om multifolliculaire groei te induceren. Nader onderzoek zal nodig zijn om de optimale dosis en het optimale toedieningsinterval vast te stellen.

### Hoofdstuk 3.1

Gonadotrofine releasing hormoon agonisten (GnRHa) worden wereldwijd gebruikt bij de ovarieële hyperstimulatie voor in vitro fertilisatie (IVF) ter preventie van een voortijdige stijging van het luteïniserend hormoon (LH). Echter, omdat de afgifte van gonadotrofinen door de hypofyse na het staken van de GnRHa, slechts zeer langzaam op gang komt, zou het gebruik van GnRHa gedurende de folliculaire fase een negatief effect kunnen hebben op de daaropvolgende luteale fase. Om die reden wordt bij IVF behandelingen altijd medicatie gegeven ter ondersteuning van de luteale fase. In dit hoofdstuk wordt een studie beschreven die werd verricht om te onderzoeken of het mogelijk was de GnRHa eerder te

stoppen zonder dat er sprake zou zijn van een voortijdige LH stijging. Daarbij was het de vraag of er -na het eerder stoppen van de GnRHa- voldoende LH zou worden afgegeven in de luteale fase om de aanmaak van progesteron door het corpus luteum te ondersteunen.

Zestig patiënten werden gerandomiseerd over 3 groepen. [1] Een lang GnRHa/human menopausal gonadotrophin (HMG) protocol met luteal support in de vorm van 4 injecties met human chorionic gonadotrophin (hCG) om de dag vanaf de dag van de punctie. (n = 20). [2] Vroeg folliculair stoppen van de GnRHa zonder luteal support (n = 20). [3] Een lang GnRHa/HMG protocol zonder luteal support (n = 20). Frequent echoscopisch onderzoek en bloedafname vonden plaats gedurende de gehele IVF cyclus. Veertig normo-ovulatoire vrouwen vormden de controle groep.

Er waren geen voortijdige LH stijgingen na het vroeg stoppen van de GnRHa. De vrouwen die eerder stopten met de GnRHa lieten wel een gering herstel zien van de gonadotrofine productie in de late luteale fase, maar dit had geen enkele invloed op de corpus luteum functie en de progesteron productie. Er werd aangetoond dat de concentratie van progesteron samenhangt met de daling van het hCG, toegediend 35 uur voor de punctie: nadat er geen hCG meer aanwezig was, verdween het progesteron. In alle groepen ontstonden zwangerschappen.

Concluderend is er na het eerder stoppen van de GnRH, nog steeds een effectieve preventie van een voortijdige LH stijging. Echter, ondanks het feit dat er een klein herstel werd gevonden in hypofyse functie leidde dit niet tot een verbeterde corpus luteum functie. Dit ten gevolge van vroegtijdige luteolysis.

### Hoofdstuk 3.2

Voor het gebruik van een GnRH agonist ter preventie van een voortijdige LH stijging tijdens ovariële hyperstimulatie voor in vitro fertilisatie is sinds kort een alternatief beschikbaar in de vorm van een GnRH antagonist. Het voordeel van het gebruik van een GnRH antagonist zou kunnen zijn dat er mogelijk geen luteale fase ondersteuning meer nodig is, omdat na het staken van een GnRH antagonist de hypofyse functie zich binnen 24 uur herstelt. In dit hoofdstuk wordt een studie beschreven die in twee IVF centra werd verricht om de luteale fase kenmerken te onderzoeken in patiënten die behandeld werden met een GnRH antagonist zonder luteal support.

De patiënten werden door loting verdeeld over 3 verschillende strategieën van laatste fase van eicel rijping. Veertig patiënten kregen ovarieële hyperstimulatie met recombinant (r-)FSH (150 IU/d vaste dagelijkse dosis) in combinatie met een GnRH antagonist (antide; 1 mg/dag) in de laat folliculaire fase. Zodra tenminste 1 follikel met een diameter van tenminste 18 mm werd gezien tijdens het echoscopisch onderzoek, werd de randomisatie verricht. De laatste fase van eicelrijping, 35 uur voor de eicelpunctie, werd geïnduceerd met een injectie met [1] r-humaan (h)CG (250 µg) (n = 11); [2], r-LH (1 mg) (n = 13) of [3] GnRH agonist (triptoreline; 0.2 mg) (n = 15). De verkregen eicellen werden bevrucht via IVF of ICSI afhankelijk van de sperma kwaliteit. Embryo transfer werd verricht

3-4 dagen na de eicelpunctie. Er werd geen medicatie ter ondersteuning van de luteale fase gegeven. Serum FSH, LH, oestradiol ( $E_2$ ), progesteron (P), en hCG spiegels werden bepaald op vaste momenten in de folliculaire en in de luteale fase.

De mediane duur van de luteale fase was respectievelijk 13, 10, and 9 dagen in de r-hCG, de r-LH, en de GnRH agonist groep ( $p = 0.005$ ). De mediane 'area under the curve' (AUC) per dag (vanaf 4 dagen na de randomisatie tot aanvang van de menstruatie) van LH was respectievelijk 0.50, 2.34, and 1.07 in de r-hCG, de r-LH, en de GnRH agonist groep ( $P = 0.001$ ). The mediane AUC per dag van P was respectievelijk 269, 41 en 16 in de r-hCG, de r-LH, en de GnRH agonist groep, ( $P < 0.001$ ). Lage zwangerschapscijfers werden gevonden in alle groepen (in alle groepen bij elkaar 7.5%; range, 0-18% per gestarte cyclus)

Concluderend bleek de niet medicamenteus ondersteunde luteale fase insufficiënt in alle groepen. Patiënten die r-hCG kregen hadden de minst verstoorde luteale fase, vergeleken met de beide andere groepen. Het is aannemelijk dat in de r-hCG groep de corpora lutea in de vroeg luteale fase nog werden ondersteund door de r-hCG bolus 35 uur voor de eicelpunctie die (door de trage klaring) gedurende meerdere dagen in de circulatie aanwezig blijft. Nadat er aanvankelijk hoge P en  $E_2$  spiegels in de vroeg luteale fase waar te nemen zijn, dalen deze spiegels extreem snel en veel te vroeg, omdat luteolyse optreedt. Deze vroegtijdige luteolyse zou het gevolg kunnen zijn van excessieve negatieve feedback door de hoge steroid spiegels op de hypofysaire LH afgifte. Het blijft dus van belang de luteale fase medicamenteus te ondersteunen bij patiënten die ovarieële hyperstimulatie krijgen in combinatie met een GnRH antagonist voor IVF

### Hoofdstuk 3.3

De luteale fase na ovariële hyperstimulatie voor IVF is insufficiënt. Vandaar dat medicatie voor de ondersteuning van de luteale fase routinematig wordt toegepast bij IVF behandelingen. De oorzaak voor de voortijdige luteolyse is echter nog niet duidelijk. Een hypothese zou kunnen zijn dat deze voortijdige luteolyse na ovariële hyperstimulatie optreedt door suprafysiologische steroid spiegels tijdens de vroeg luteale fase. In de studie die in dit hoofdstuk wordt beschreven, worden hoge doseringen steroiden toegediend na de LH piek in normo-ovulatoire vrouwen. De studie werd in deze vrijwilligers verricht teneinde te onderzoeken of deze interventie endocriene veranderingen zou bewerkstelligen en of er sprake zou zijn van een luteale fase.

Veertig vrouwen met een normaal gewicht tussen de 18 en 37 jaar met een reguliere menstruele cyclus (24–35 d) die niet roken werden gerandomiseerd voor hoge doseringen  $E_2$ , P,  $E_2 + P$  of geen medicatie. Vanaf de dag van de LH piek tot en met LH +14 werd bloedafname verricht. De duur van de luteale fase en hormonale spiegels waren de belangrijkste uitkomstmaten voor dit onderzoek.

De vroeg luteale steroid concentraties die verkregen werden na toediening van de studie medicatie, waren vergelijkbaar met de spiegels zoals die beschreven werden bij patiënten die een ovariële hyperstimulatie voor een IVF behandeling hadden ondergaan.

Er werd geen verschil in duur van de luteale fase geobserveerd. Maar bij de vrouwen die P toegediend hadden gekregen, werd wel een significante verlaging van LH spiegels gevonden op dag 6 na de LH piek ( $p < 0.001$ ). Deze tijdelijke verlaging in LH resulteerde in versnelde daling van de inhibine A productie door het corpus luteum ( $p = 0.001$ ).

Concluderend was er geen sprake verkorting van de luteale fase na toediening van hoge doseringen steroïden, kort na de LH piek, in vrouwen met een normo-ovulatoire cyclus. Blijkbaar kon de corpus luteum functie zich herstellen van de kortdurende daling van LH. Het zou mogelijk kunnen zijn dat tevens andere factoren een rol spelen bij de uitgesproken luteale fase duur verkorting na ovarieële hyperstimulatie voor IVF.

### **Hoofdstuk 4**

Dit hoofdstuk is een brede samenvatting van de conclusies die uit de beschreven studies getrokken kan worden.

## **Publications and presentations**

### **Publications included in this thesis**

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## Dankwoord

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## **Curriculum vitae auctoris**

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De auteur van dit proefschrift, Nicole Geertje Maria Beckers werd geboren op 25 februari 1964 te Den Haag. In 1982 behaalde zij het eindexamen Atheneum aan het Niels Stensen College te Utrecht. In mei 1990 behaalde zij het artsexamen aan de Erasmus Universiteit te Rotterdam. Vanaf mei 1990 was zij gedurende 1 jaar werkzaam als arts-assistent/poortarts in het Hofpoort Ziekenhuis te Woerden. Aansluitend werkte zij een aantal maanden als arts-assistent niet in opleiding op de afdeling Gynaecologie en Verloskunde in het Kennemergasthuis te Haarlem. Vanaf november 1991 is zij werkzaam als fertiliteitsarts op de subafdeling Voortplantingsgeneeskunde afdeling Verloskunde en Vrouwenziekten van ErasmusMC te Rotterdam.

