Summary
The optimal gonadotropin releasing hormone (GnRH) antagonist regimen has not been elucidated yet, despite over 200 reported clinical studies using GnRH antagonists. Currently available meta-analysis included all comparative studies between GnRH agonists and antagonists performed so far, including less than optimal GnRH antagonist regimens leading to conflicting results dependent on the selection of studies made. The aim of this thesis was to search for the optimal GnRH antagonist regimen and to compare it with GnRH agonists for its use in in-vitro fertilization (IVF). As outlined in chapter 1, the studies reported in the first part of this thesis (chapter 2 to 8) aim to search for factors which may improve GnRH antagonist regimen in terms of follicular development and oocyte yield. The studies in the second part of this thesis (chapter 8 and 9) aim to search for the optimal comparison of GnRH antagonists with GnRH agonists to evaluate the current place of GnRH antagonists in IVF.

CONSIDERATIONS IN SELECTING THE OPTIMAL GNRH ANTAGONIST REGIMEN

Chapter 2
This chapter addresses the development of GnRH antagonists, their mechanism of action their possible clinical applications in the field of reproduction and beyond.

Pulsatile GnRH stimulates the pituitary secretion of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) and thus controls the hormonal and reproductive function of the gonads. Blockade of GnRH effects may be wanted for a variety of reasons, e.g. to prevent untimely luteinization during assisted reproduction or in the treatment of sex-hormone-dependent disorders. Selective blockade of LH/FSH secretion and subsequent chemical castration have previously been achieved by desensitizing the pituitary to continuously administered GnRH or by giving long-acting GnRH agonists.

Only recently have GnRH receptor antagonists, that immediately block GnRH’s effects, been developed for clinical use with acceptable pharmacokinetic, safety, and commercial profiles. We concluded that all current indications for GnRH agonist desensitization may prove to be indications for a GnRH antagonist, including endometriosis, leiomyoma, breast cancer, benign prostatic hypertrophy and prostatic carcinoma and central precocious puberty. However, the best clinical evidence so far has been in assisted reproduction and prostate cancer. Finally, licensed GnRH antagonists are ideal research tools for further exploration of the pathophysiology of the human reproductive system.

Chapter 3
This chapter provides an overview of the literature with respect to various drugs and therapeutic regimens (i.e. GnRH agonists and antagonists, urinary and recombinant gonadotrophins) used for ovarian stimulation in IVF.

FSH treatment to induce follicular development in an-ovulating women and multiple follicular development for assisted conception has been incorporated in almost all reproductive treatment cycles in the form of either urinary, purified urinary or recombinant preparations. Besides improved tolerance and theoretically lower chances of infection by prions, the latter have proven to be more effective in terms of clinical pregnancy rates, FSH requirement and cost effectiveness. The low-dose step-up protocol to induce monofollicular development, which is applied worldwide, has to compete with the equally effective but
health economically beneficial step-down protocol. The long protocol using recombinant FSH 150 IU/day is advocated when using GnRH agonists in IVF or intracytoplasmatic sperm injection (ICSI) treatment. Besides this well adopted strategy, GnRH antagonists with acceptable safety profile became available which allow alternative treatment strategies with milder approaches with acceptable cancellation rates. Additionally GnRH antagonists allow the use of GnRH agonists to trigger final oocyte maturation and ovulation; the latter require pituitary responsiveness and are therefore excluded in agonist protocols. FSH and LH are both required for appropriate folliculo- and steroidogenesis. In hypogonadotropic women, the addition of LH (human menopausal gonadotrophin, human chorionic gonadotrophin or rLH) is therefore obligatory to achieve appropriate follicular growth and pregnancy. The role of LH in ovulation induction is still a matter of debate, although in GnRH agonistic protocols there seems to be a ‘therapeutic window’; levels that are too high or too low have detrimental effects on IVF outcome. Prospective studies investigating possible direct effects of GnRH analogues, optimal dose-finding studies and treatment regimens under different conditions, with or without pharmacological co-administration and for different indications, should be performed to optimise the efficacy and tailor treatment strategies to individual needs.

Chapter 4
The aim of this chapter was to define the minimal effective dose of a new GnRH antagonist antide to suppress LH levels and to prevent premature LH surges in IVF patients.

We performed a prospective parallel, single centre study. The primary endpoint of this study was to determine the minimal effective dose, defined as the lowest dose group in which fewer than two LH surges occurred. Secondary endpoints were drug requirements, serum hormone and antide concentrations and safety aspects. This study was conducted in two phases, a double-blind phase (n=60) was followed by an open phase in which 3 additional treatment groups were added with lower antide dosages. In total 144 IVF/ICSI patients were stimulated with rhFSH from cycle day 2 onwards, and co-treated with daily 2 mg/2 ml (n=30), 1 mg/ml (n=30), 0.5 mg/ml (n=31), 0.5 mg/0.5 ml (n=23) and 0.25 mg/ml (n=30) GnRH antagonist (antide) from cycle day 7 onwards.

Serum samples were taken three times daily during antide administration to assess antide and hormone levels. Serum antide levels, mean LH and oestradiol levels per day and their area under the curves were dose-related to antide. The bioavailability of antide almost doubled after dilution in larger volumes. LH levels immediately decreased after the first antide injection, pre-injection LH levels gradually increased during GnRH antagonist treatment. LH surges occurred only in the lowest dose groups 0.5 mg/ml (3.2%), 0.5 mg/0.5 ml (6.7%) and 0.25 mg/ml (13.3%). Hence, 0.5 mg/ml is considered to be the minimal effective dose. Antide was overall well tolerated and safe. We concluded that 0.5 mg/ml antide is the minimal effective dose to prevent an untimely LH surge in IVF patients stimulated with rhFSH.

Chapter 5
This chapter addresses the relation between various LH and progesterone concentrations, induced by different GnRH antagonist doses, on IVF outcome.

We performed a prospective, single centre study including 144 IVF patients, stimulated with recombinant FSH from cycle day 2, and co-treated with daily GnRH antagonist
Summary

(antide) (2 mg/2 ml, 1 mg/ml, 0.5 mg/ml, 0.5 mg/0.5 ml or 0.25 mg/ml) from cycle day 7 onwards. Serum samples were taken three times daily. Outcome measures were drug requirements, stimulation results, IVF outcome and its relationship to serum and antide concentrations. There were no differences in number of mature follicles (≥11 mm), mean follicular size and endometrial thickness on the day of hCG administration between the various dose groups. Also no differences were found in number of oocytes retrieved, mean number of metaphase II oocytes per patient, fertilization rate, total number of embryos, good quality embryos (grade I and II) and number of embryos transferred. There was a tendency toward higher pregnancy and implantation rates in the middle dose groups (0.5 and 1.0 mg/ml; not significant). None of the patients in this study had moderate or severe symptoms associated with OHSS necessitating hospitalization. Clinical pregnancies were only observed within a particular range of change in LH levels.

Based on these results we concluded that excessive or insufficient suppression of LH and progesterone levels during GnRH antagonist administration and high progesterone per follicle on the day of hCG administration seems to be associated with impaired clinical pregnancy rates.

Chapter 6
The aim of this chapter was to assess the effect of oral contraceptive (OC) pretreatment in a fixed GnRH antagonist IVF protocol on the coordination of follicular development and the number of oocytes retrieved, in comparison to a control group without OCs.

To address this issue, we randomized 64 IVF patients to start rhFSH on day 2 or 3 after OC withdrawal (OC group) or on day 2 of a natural cycle (control group). From stimulation day 6 onwards, all patients were treated with daily (0.5 mg/ml) GnRH antagonist (antide). The primary efficacy endpoint was the number of oocytes retrieved. OC pretreatment resulted in significantly lower starting concentrations of FSH, LH and oestradiol and a thinner endometrium. In the early stimulation period, fewer large follicles were found after OC pre-treatment, leading to an extended stimulation period (11.6 versus 8.7 days, p<0.0001) with more follicles on the day of rhCG administration (15.4 versus 12.5, p=0.02) and more oocytes retrieved (13.5 versus 10.2, p<0.001) as compared with the control group. Based on these results we concluded that OCs pretreatment in a fixed GnRH antagonist regimen in which rhFSH is started 2 days after the last OCs, improved follicular homogeneity with an extended stimulation period, more rhFSH required and more oocytes retrieved.

Chapter 7
Adresses the effect of timing gonadotrophin administration (day 2 versus day 5) after the last oral contraceptives (OCs) taken, with or without rLH addition, in a fixed GnRH antagonist protocol on hormonal concentrations and follicular development.

We randomized 48 IVF/ICSI patients treated with a fixed day 6, GnRH antagonist protocol, to start with daily 150 IU rhFSH 2 or 5 days after last OCs taken, with or without addition of 150 IU rhLH/day. The primary efficacy endpoint was the number of mature follicles (≥11 mm) on the day of hCG administration. Starting on day 2 versus day 5 leads to significantly lower starting concentrations of FSH, LH and oestradiol, a thinner endometrium (p<0.001) and less large follicles on S6, an extended stimulation period (8.0 versus 4.8 days, p<0.001) and more rhFSH required (1981 and 1474 IU, respectively) (p<0.001). Hormonal concentrations and number of oocytes inseminated were similar on the day of hCG administration (hCGd).
Addition of daily rhLH leads to higher LH concentrations (all >1.0 IU/L), less large follicles on hCGd and a lower number of oocytes available for insemination (p<0.001).

In conclusion, although the follicular cohort is more uniformly developed if gonadotrophins administration is started 2 versus 5 days after OC withdrawal, this does not lead to an improved oocyte yield despite more administered gonadotrophins. Addition of 150 IU rhLH per day in OC pretreated GnRH antagonist regimen results in higher LH levels but may produce less follicles and oocytes.

Based on the results of this small study we postulate that starting with gonadotrophins 5 days after OC withdrawal is to be preferred in view of shorter treatment and less gonadotrophin requirements and preferentially without LH addition.

SEARCHING FOR THE OPTIMAL COMPARISON OF GNRH ANTAGONISTS AND AGONISTS

Chapter 8
The aim of this chapter was to compare the oocyte yield and IVF outcome in patients treated with a long GnRH agonist regimen and an oral contraceptive (OC) pretreated fixed day 6 GnRH antagonist protocol in which stimulation was started on day 5 after last OCs.

To address this aim, we performed a multicentre, randomized study. 182 patients were randomized to receive cetrorelix with OC pretreatment (n=91) or to receive buserelin (n=91). The cetrorelix group started with daily OCs on cycle day 5, during 21 to 28 days. Cetrorelix (0.25 mg) was given daily from stimulation day 6 up to and including the day of rhCG administration. The buserelin group started with buserelin (500 μg/day) for at least 10 days until down-regulation was achieved, after which the dose was reduced to daily 200 μg up to and including the day of rhCG administration. RhFSH was started in both groups on a Friday, in the cetrorelix group 5 days after last OC intake. Both regimens were followed by a general IVF or ICSI procedure. The primary efficacy endpoint was the number of oocytes retrieved per patient. Number of oocytes, cancellation rates, rhFSH requirements, number of ovum pick-ups (OPU) in the weekend or public holiday and number of pregnancies were similar in both groups. Both treatment regimens were well tolerated.

In conclusion, it seems that the combination of OCs with antagonist largely overcomes several disadvantages of GnRH antagonist application in IVF that were claimed in the past compared with long agonist regimens. The mean number of oocytes retrieved were similar in OC pretreated cetrorelix group and the long buserelin group with only a small number of OPU on weekend or public holidays. In our opinion this combined strategy, in particular given the significant reduction of required injections and in part as result of that significant reduction in reported side effects before stimulation, provides a good alternative for the long GnRH agonist protocol in prevention of premature luteinization in IVF.

Chapter 9
The aim of this chapter was to identify the optimal GnRH antagonist regimen in terms of IVF outcome and scheduling ability, to compare with GnRH agonists.

To establish this we reviewed currently available literature with respect to various GnRH antagonist regimens and comparative studies including GnRH agonists and
antagonists in IVF. We believe that appropriate comparison of optimal GnRH agonist and antagonist regimens has not been performed yet. Currently available meta-analysis included all comparative studies between GnRH agonists and antagonists performed so far, including less than optimal GnRH antagonist regimens. After critical appraisal of the various studied GnRH antagonist regimens in terms of follicular development and IVF outcome, we postulate that early suppression of endogenous FSH results in optimal follicular development. Additionally, stable and early suppression of LH and progesterone levels during the entire period of stimulation may be an advantage for implantation and pregnancy outcome. In this respect, single dose and particularly flexible protocols seem to be less advantageous. LH addition does not offer benefits in terms of pregnancy rates in a general IVF population. Early FSH and LH suppression can be achieved by early GnRH antagonist administration (stimulation day 1) or by OC pretreatment.

A meta-analysis including four studies comparing the long GnRH agonist protocol with OC-pretreated fixed GnRH antagonist regimen; could not identify differences in number of oocytes retrieved and clinical pregnancies. We stress that it is too early to denigrate the GnRH antagonist before the optimal GnRH antagonist protocol has been compared with the optimal GnRH agonist regimen in a prospective randomized fashion. More studies comparing long GnRH agonist protocols with “long” GnRH antagonist protocols, with enough power to identify differences in pregnancy rates, (long or pretreated with oestrogens or oral contraceptives) are required before appropriate comparison can be made.