DECREMENTAL FOLLICLE-STIMULATING HORMONE AND SINGLE DOMINANT FOLLICLE SELECTION IN THE HUMAN

DALEND FOLLIKEL-STIMULEREND HORMOON EN SELECTIE VAN ÉÉN DOMINANTE FOLLIKEL BIJ DE MENS

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List of Abbreviations

AD	androstenedione
BMI	body mass index (= weight / height^2)
CRA	clomiphene-citrate resistant anovulation
DHEAS	dehydroepiandrosterone sulphate
E_2	17β-estradiol
FAI	free androgen index (= T x 100 / SHBG)
FSH	follicle-stimulating hormone
GnRH	gonadotropin releasing hormone
hCG	human chorionic gonadotropin
HMG	human menopausal gonadotropin
im	intramuscular
IRMA	immunoradiometric assay
iv	intravenous
IVF	*in-vitro* fertilization
LH	luteinizing hormone
OHSS	ovarian hyperstimulation syndrome
P	progesterone
PCO	polycystic ovaries
PCOS	polycystic ovary syndrome
pFSH	purified urinary follicle-stimulating hormone
RIA	radioimmunoassay
sc	subcutaneous
SD	standard deviation
SHBG	sex-hormone binding globulin
T	testosterone
TSH	thyroid stimulating hormone
TVS	transvaginal sonography
WHO	World Health Organization

1

Introduction

1.1 HISTORY AND DEFINITION OF STUDY OBJECTIVES

One of the earliest known anatomical descriptions of the ovaries is produced by Soranus of Ephesus (AD 98–138) who had been studying in Alexandria and practiced in Rome. During the following Middle Ages, no substantial progress was made regarding reproductive anatomy until Leonardo da Vinci (1452–1519), who accurately drew the anatomy of the human uterus and ovaries. In the 16th century, the University of Padua was an important center of research concerning human anatomy, and produced famous names like Vesalius and Fallopius. Vesalius (1515–1564) described the hypophysis as the 'glandula pituitaria cerebri excipiens' and was the first to recognize follicles in the human ovary. Reinier de Graaf (1641–1673), a Dutch physician born in Delft, was the first to describe the 'female testis' containing the ovarian follicles. Subsequently, investigators referred to the mature dominant follicle as the 'Graafian follicle'. In the beginning of the 20th century animal studies were conducted in which pituitary extracts and urine of pregnant women were injected into rats (Smith and Engle, 1927; Aschheim and Zondek, 1927). These animal studies resulted in recognition of two pituitary hormones (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]) with different action in the ovary (Fevold *et al.*, 1933). It also became clear that urine of pregnant women contained predominantly a luteinizing hormone of placental origin, whereas urine of climacteric women could induce follicle growth in the ovary (Leonard and Smith, 1934). When it finally became possible to extract gonadotropins from pregnant mare serum, a problem in clinical application arose: anti-gonadotropin antibody formation. A proper way of extracting the FSH-like substance from the urine of postmenopausal women was finally proven to be gel-electrophoresis (Borth *et al.*, 1957). This purification technique facilitated the production of human gonadotropins and its wide-scale clinical use as treatment for patients with oligo- or amenorrhea. Initially, detailed knowledge regarding the structure and function of the ovary was derived from morphological studies, while the introduction of pelvic ultrasonography (Hackeloer *et al.*, 1979), made it possible to study the dynamics of ovarian follicle develop-

ment *in vivo*. Although this new imaging technique represented a major step to a better understanding of the dynamics of follicle growth it was not until transvaginal ultrasonography was introduced, allowing the use of high frequency probes in close proximity to the ovaries, that follicles as small as 2 mm in diameter could be visualized (Pache *et al.*, 1990). On the other hand, development of steroid and peptide assays in serum or follicular fluid, and *in vitro* cultures of human ovarian cells increased our knowledge of endocrine and para/autocrine factors regulating follicle development in the human ovary (Schoot *et al.*, 1992a; Fauser *et al.*, 1995).

Anovulation represents a major cause of female infertility. Menstrual cycle disturbances usually are a reflection of hormonal imbalance. Patients presenting with oligomenorrhea or amenorrhea are categorized by the WHO (World Health Organization Manual, 1993) into 3 classes, based upon serum hormone assays in peripheral blood: Class I, hypogonadotropic hypoestrogenic anovulation; Class II, normogonadotropic normoestrogenic anovulation; and Class III, hypergonadotropic hypoestrogenic anovulation. Important clinical conditions include premature ovarian failure (Class III), as well as chronic anovulation in patients presenting with serum FSH and estradiol in the normal range, polycystic ovary syndrome (PCOS). PCOS, the most common anovulatory disorder in women, is often characterized by hirsutism, obesity and oligo- or amenorrhea together with disrupted follicle growth in enlarged ovaries, raised serum concentrations of circulating androgens and elevated serum LH concentrations. Because not all criteria are mandatory, the PCOS patient group is very heterogeneous. This may be due to the fact that multiple defects described in the PCOS population may result in a similar endpoint: polycystic ovaries. The first description by Stein and Leventhal in 1935 (Stein and Leventhal, 1935), was based upon a specific medical history and characteristic findings upon physical examination. However, the major finding was the polycystic morphology of the ovaries: 'two to four times the normal size, often maintaining their original shape, but sometimes globular or flattened like an oyster. Cortex was hypertrophied, tunica was thickened, tough, and fibrotic. Cysts were follicular cysts nearly all confined to the cortex, and contained clear fluid. There were from 20 to 100 in each ovary, varying in size from 1 mm to about 15 mm, rarely larger. The color of the ovary was oyster-gray with bluish areas where the cysts were superficial'. More recently, diagnosis of PCOS was merely based on clinical symptoms and biochemical parameters. At present, some investigators apply just a single biochemical marker for PCOS diagnosis, such as elevated serum LH concentration, whereas others use only sonographic parameters or a combination of these two. Presently, most clinical investigators seem to

consider the ultrasound appearance of polycystic ovaries of reduced significance for PCOS diagnosis. This approach could be criticized on the basis of the historical description of the syndrome, as mentioned above. Correct and strict diagnosis of PCOS and division in subgroups may be relevant because of its possible future implications regarding decreased pregnancy chances, augmented complication rates during induction of ovulation (prediction of treatment outcome), and increased long-term health risks (for instance cardiovascular disease or diabetes).

Induction of ovulation using exogenous gonadotropins in women presenting with chronic anovulation is a widely used treatment modality since the 1960s. The great majority of anovulatory patients presently treated with gonadotropin preparations comprise normogonadotropic anovulatory infertile patients (WHO group II) who failed to ovulate (or conceive) during previous antiestrogen medication. Although gonadotropin therapy has been shown to be fairly successful in terms of ovulation rates (reported in literature between 60–100%) and cumulative pregnancy rates (reported between 20–75%), complication rates are high. Major complications include ovarian hyperstimulation (Navot *et al.*, 1992), multiple pregnancies (Derom *et al.*, 1993) and a high incidence of early pregnancy wastage (Shoham *et al.*, 1991).

In this thesis we would like to focus on patients suffering from normogonadotropic anovulatory infertility (WHO, class II) and normo-ovulatory controls. This concerns a major proportion of the infertility population (20–25%). The objective of this thesis is to generate more insight into (patho)physiological mechanisms concerning dynamics of follicle growth in the human ovary, using frequent transvaginal ultrasonography (TVS) combined with hormonal serum assays in peripheral blood. The process of follicle recruitment and selection will be closely studied during the follicular phase of the normal menstrual cycle and during induction of ovulation using gonadotropins, whereas the role of decreasing serum FSH concentrations will be especially evaluated.

1.2 OVARIAN PHYSIOLOGY

1.2.1 Morphology of the human ovary

During the second month of fetal life differentiation of the indifferent gonads takes place. Three different regions develop: the rete ovarii or hilum, the central medullary portion and an outer cortical portion. The hilum contains nerves, blood vessels and cells that secrete steroid hormone; the cortical epithelium consists of an unicellular layer of cuboidal or squamous cells and the inner part contains stroma (connective tissue, contractile cells and interstitial cells) and immature germ cells – follicles – enclosed

in cellular complexes. Between weeks 4 and 6 of gestation the primordial germ cells have migrated from the primitive ectoderm at the caudal end and the yolk sac to the gonadal sites. During their movement they begin their proliferation. By the sixth week, on completion of the indifferent state, these primordial germ cells have multiplied by mitosis to a total of 10 000. At 6–8 weeks, the first signs of ovarian differentiation are reflected in the rapid mitotic multiplication of germ cells, reaching 6–7 million oogonia by 16–20 weeks (Baker *et al.*, 1963). This represents the maximal oogonal content of the gonad. From this point in time germ cell content will irretrievably decrease until, some 50 years later, the store of oocytes will finally be exhausted. The oogonia are transformed to oocytes as they enter the first meiotic division and arrest in prophase. This process begins at 11–12 weeks, perhaps in response to factors produced by the rete ovarii. Progression of meiosis to the diplotene stage is accomplished throughout the rest of pregnancy and completed by birth. There is a temporary arrest in oocyte development, characterized by an oocyte in prophase of the first meiotic division enveloped by a single layer of granulosa cells surrounded by a basement membrane; this is called the primordial follicle.

1.2.2 Gonadotropin-independent early follicle development

A continuous flow of primordial follicles from a resting to a growing stage can be observed throughout life. During the first period, follicle depletion starts at a very high rate, and approximately 2 million primordial follicles remain at birth (Peters, 1979). After birth this process slows down. When reproductive life starts at menarche, approximately 0.5 million primordial follicles are present (Hillier *et al.*, 1981). During the reproductive period follicle-loss takes place at a fixed-rate of approximately 1000 follicles per month, accelerating beyond the age of 35 until no more follicles are left around menopause (Faddy *et al.*, 1992; Gougeon, 1993). Factors involved in regulation of primordial follicle growth are still largely unknown. Once follicles are stimulated to grow they can either become dominant follicles and ovulate or become atretic. Follicles are present in the ovary at different stages of development with different sizes at any point during the menstrual cycle (Gougeon, 1986).

The primordial follicle is characterized by an oocyte in the prophase of the first meiotic division, surrounded by a layer of granulosa cells. Initiation of growth results in growth of the oocyte and granulosa cell proliferation (primary follicle). The secondary cell stage involves a division of stroma in a theca externa and a theca interna. Theca interna cells exhibit LH receptor activity early on (Channing *et al.*, 1973). During the development of an antral-cavity, granulosa cells containing FSH-receptors have been ob-

served, as cumulus cells surrounding the oocyte and cells bordering the basement membrane (Gougeon, 1993). It remains controversial whether or not pre-antral follicle development is totally independent from FSH (Oktay *et al.*, 1997). After antrum formation has occurred, FSH is needed for ongoing follicle maturation although the exact time of initiation of gonadotropin-dependent follicle maturation is still uncertain. Complete follicle maturation takes at least 85 days (Gougeon, 1996). In a lifetime, only 400 follicles reach full maturation and ovulate, leaving the remaining follicles to a process referred to as apoptosis ('programmed cell-death'). Survival factors suppressing this process are gonadotropins and growth factors (Billig *et al.*, 1993; Hsueh *et al.*, 1994), while oxidative stress induces atresia (Tilly *et al.*, 1995). FSH decreases apoptosis in granulosa cells obtained from hypophysectomized rats (Billig *et al.*, 1994) and prevents apoptotic changes of cultured preovulatory follicles (Chun *et al.*, 1994).

1.2.3 Gonadotropin-dependent advanced follicle development

Ongoing follicle growth from the antral stage is not possible without gonadotropins. Both gonadotropins, LH and FSH produced by the anterior pituitary, have a synergistic action during follicle development. Theca cells are stimulated by LH to convert cholesterol to androstenedione (AD) and testosterone (T) by cytochrome p450 oxidases and 3β-OH-steroid dehydrogenase, whereas granulosa cells aromatase activity (cytochrome p450 aromatase) is induced by FSH and converts these androgens (AD and T) to estrone and estradiol (E_2). The concept of two different cell-types (granulosa and theca cells) and two hormones (LH and FSH) necessary to convert cholesterol to estrogens is referred to as the 'two-cell, two-gonadotropin' theory. FSH enhances aromatase activity in granulosa cells, induces LH and increases FSH receptor formation and stimulates general cell functions such as DNA and protein synthesis (Hsueh *et al.*, 1989).

Two theories are held responsible for the development of a single ovarian follicle (Figure 1): the 'FSH-threshold' (Brown, 1978; Schoemaker, 1993) and 'FSH-window' (Baird, 1987; Fauser *et al.*, 1993a; Fauser, 1994) theory. During the luteo-follicular transition when FSH concentrations start to rise, a cohort of 'receptive' follicles is recruited. Each individual follicle in the cohort of recruited follicles has its own specific FSH-threshold that should be surpassed to start gonadotropin dependent pre-ovulatory growth. In case the FSH-threshold is not surpassed these follicles will go into atresia. The FSH-threshold of a given follicle is not fixed but dependent on its developmental stage. The time-period during which the FSH-threshold is surpassed, the FSH window, determines the

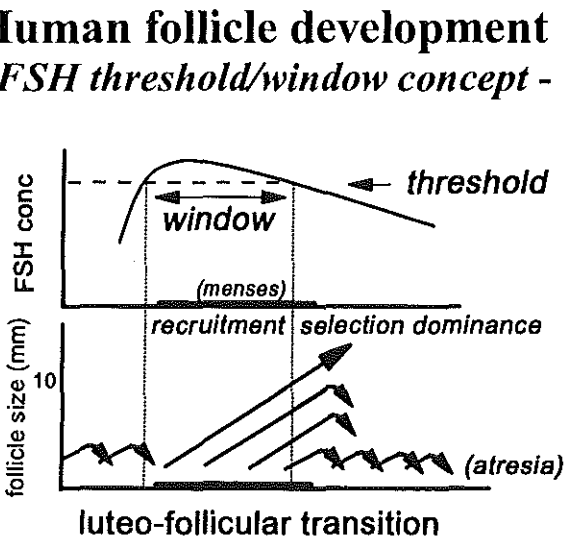

Figure 1 Schematic representation of the intercycle rise in serum FSH concentrations (FSH threshold/window concept), and follicle growth dynamics (recruitment, selection, and dominance) during the follicular phase of the normal menstrual cycle. [Reproduced with permission from Fauser, B.C., and van Heusden, A.M. (1997a). Manipulation of human ovarian function. *Endocr Rev* 18, 71-106].

number of follicles that are recruited to start preovulatory growth. The moment the follicle begins preovulatory development (dominance) it will gradually become less dependent on FSH and will start to produce large amounts of E_2. Plasma E_2 and inhibin B concentrations rise and subsequently serum FSH concentrations decrease while follicle growth continues at a constant rate until ovulation takes place. Proof of the concept that throughout the menstrual cycle recruitable follicles are present, has been delivered by previous human studies in which the dominant follicle has been removed at the end of the follicular phase, or luteectomy in the luteal phase was performed resulting in new follicle recruitment and subsequent ovulation (Nilsson *et al.*, 1982; Araki *et al.*, 1983; Baird *et al.*, 1984). Exact mechanisms underlying selection of the dominant follicle and subsequent monofollicular growth are still unknown, but diminished stimulation of non-dominant follicles by relatively low FSH concentrations in combination with local upregulation of FSH action in the dominant follicle might play an important role (Hodgen, 1982).

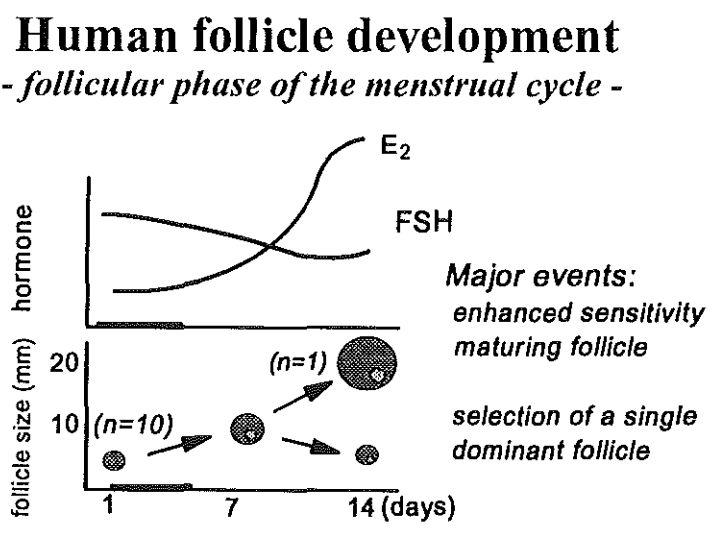

Figure 2 Schematic representation of patterns of serum FSH and E_2 concentrations, and gonadotropin dependent follicle growth, resulting in selection of a single dominant follicle during the follicular phase of the normal menstrual cycle.

1.3 DYNAMICS DURING THE NORMAL MENSTRUAL CYCLE: THE ENDOCRINE CONCEPT

During the luteo-follicular transition, a rise in serum FSH is induced on the 12th day after the preceding LH-surge (Hall *et al.*, 1992) by decreasing corpus luteum steroid production. Selection of a cohort of follicles takes place, referred to as 'recruitment' (Goodman *et al.*, 1983), when FSH surpasses its threshold. From this cohort of follicles selection of a single dominant follicle takes place. This process may be regulated by decreasing serum FSH concentrations in combination with increased sensitivity for FSH of the selected follicle (Figure 2). FSH stimulation of the selected follicle may be modulated by FSH isoforms (Chappel, 1995; Fauser *et al.*, 1997), interference with FSH receptor binding, or postreceptor signal transduction (Padmanabhan *et al.*, 1988; Fauser, 1996). The dominant follicle starts to produce E_2 from a 10 mm stage (Hillier *et al.*, 1980; van Dessel *et al.*, 1996). Serum E_2 concentrations rise and mediate negative feed-back at the pituitary and hypothalamic level, resulting in a further decrease of serum FSH concentrations. The role of inhibin in dominant follicle selection is still unclear, although it may be proposed that the rapid rise in inhibin B just after the intercycle FSH rise, which shortens the duration of the FSH-rise (narrows the window), is crucial for single dominant follicle development (Groome *et al.*, 1996). Whereas the domi-

nant follicle becomes less dependent on FSH and continues to grow (van Santbrink *et al.*, 1995a), the FSH threshold will not be surpassed for less mature follicles from the recruited cohort resulting in atresia. Induction of the LH surge by increasing serum E_2 concentrations (Fritz *et al.*, 1992), results in ovulation and formation of the corpus luteum. Production of progesterone and E_2 by the corpus luteum inhibits gonadotropin synthesis by the pituitary, but when the corpus luteum undergoes atresia in the absence of pregnancy a new menstrual cycle is initiated.

Various forms of FSH are synthesized and secreted by the anterior pituitary, on the basis of differences in oligosaccharide structure of these glycoproteins as well as the number of incorporated terminal sialic-acid residues. FSH heterogeneity should be considered as a continuum of molecular forms, each with distinct physiochemical characteristics. Heavily sialated (more acidic) FSH has been described to exhibit reduced receptor binding and *in vitro* bioactivity, whereas circulating half-life of these forms is extended. In contrast, basic isoforms have been described to be more biopotent *in vitro* (2- to 5-fold), whereas the circulating half-life is significantly reduced (Chappel, 1995). Estimates of changes in FSH heterogeneity, as assessed by *in vitro* bioassays, during the menstrual cycle are contradictory (Jia *et al.*, 1986; Padmanabhan *et al.*, 1988; Reddi *et al.*, 1990) and appear to be dependent on the assay system used. It has been speculated that ovarian follicles are recruited in the early follicular phase (when gonadal feedback is low) predominantly by more acidic FSH isoforms, whereas follicle selection and rupture later during the follicular phase is dependent chiefly on more basic FSH isoforms. However, the effect of a predominance of more bioactive but shortened half-life forms on the overall *in vivo* biopotency is unknown at this stage, and therefore the physiological significance of described changes in FSH isoforms remains open for speculation (Fauser *et al.*, 1997a; Fauser *et al.*, 1997b).

1.4 INTRA-OVARIAN MODIFICATION OF FSH ACTION: THE AUTOCRINE / PARACRINE CONCEPT

Besides systemic regulation of FSH action mediated by the hypothalamo-pituitary axis, local intraovarian regulation of FSH action also takes place. Differences in distribution of various FSH isoforms may affect FSH-receptor interaction. Moreover, interference with post-receptor signal transduction by growth-factors may also modulate the FSH signal at the ovarian level.

During the follicular phase the dominant follicle continues to mature despite decreased stimulation by lower late follicular phase FSH serum concentrations (v Santbrink *et al.*, 1995a). This observation of decreased

dependency of the dominant follicle on FSH stimulation strongly suggests that the FSH signal is modified within the ovary, either at the level of FSH binding to the receptor or by interference with the post receptor signal transduction. In addition, the intrafollicular rise in E_2 concentrations of the dominant follicle was believed to be responsible for the decreased need for stimulation by FSH through autocrine short-loop up-regulation (Hsueh *et al.*, 1983). However, it is now clear that follicles can mature fully without a concomitant rise in E_2 (Schoot *et al.*, 1992a). This observation strongly suggests that in fact other (intraovarian) factors drive growth of the dominant follicle, and disturbed intraovarian regulation may prove to be crucially important for cessation of follicle development in PCOS patients. Moreover, a 2.5-fold difference in maximum early follicular phase serum FSH concentrations – not correlated with cycle characteristics, or age, follicular phase duration or follicle growth (van Santbrink *et al.*, 1995a; Schipper *et al.*, 1998a) – observed in a group of young women presenting with normal ovarian function suggests distinct differences in intraovarian regulation under normal conditions. Support for an autocrine / paracrine role of the intraovarian insulin-like growth factors (IGF) system has been observed in a study performed by our group (van Dessel *et al.*, 1996) in which the IGF-binding protein profile in follicular fluid did change during follicle development in the normal cycle, independent of changes in serum. Various factors like the growth factor systems (IGF system, IGF-binding protein system, epidermal growth factors, and the inhibin/activin system) have been shown to affect follicle development and FSH action *in vitro* (Figure 3), but conclusive evidence for a distinct role *in vivo* is still lacking.

1.5 GONADOTROPIN INDUCTION OF OVULATION

After primary treatment of normogonadotropic oligo- or anovulatory infertility with clomiphene-citrate, 30% of these patients will remain anovulatory and only 50% of women will conceive (Gorlitsky *et al.*, 1978; Imani *et al.*, 1998). For many years second line therapy for this poorly defined patient group is gonadotropin induction of ovulation. Although indisputably effective, these compounds carry important risks; chiefly ovarian hyperstimulation (Stephenson, 1991; Navot *et al.*, 1992) reported in literature to occur between 3%–15% of patients (Fauser *et al.*, 1997a) and multiple pregnancies (Derom *et al.*, 1993) reported in literature to occur between 4%–28% of patients (Fauser *et al.*, 1997a) associated with considerable obstetrical complications (Levene *et al.*, 1992; Callahan *et al.*, 1994). In an attempt to reduce complication rates various treatment schedules have been developed. Schedules reported initially include single dose, intermittent or multiple fixed doses and incremental or decremental

Enhanced sensitivity for FSH of the maturing follicle

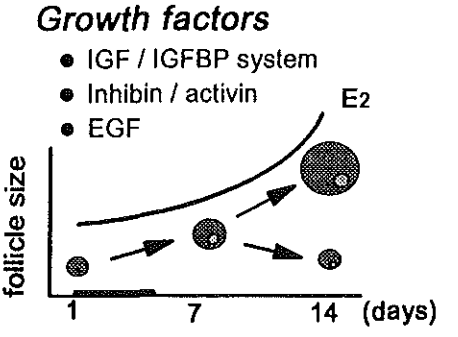

Figure 3 Schematic representation of growth factors potentially involved in late follicular phase enhancement of FSH action. [Reproduced with permission from Fauser, B.C., and van Heusden, A.M. (1997a). Manipulation of human ovarian function. *Endocr Rev* 18, 71-106.

dose regimens (Taymor *et al.*, 1967; Thompson *et al.*, 1970). At present, most frequently used administration schedules are 'step-up' regimens; i.e. increasing doses of exogenous gonadotropins are administered through daily intramuscular injections until ovarian response is considered to be 'sufficient'. In an attempt to diminish complication rates, conventional step-up regimens were modified to 'low dose, step-up' regimens (Seibel *et al.*, 1984; Buvat *et al.*, 1989; Hamilton-Fairley *et al.*, 1991) starting with a lower initial dose (½ or 1 ampule per day) and with small subsequent increments (½ ampule) at weekly intervals in case of absent response. With the use of low-dose regimens improved clinical outcome has been reported. There were lower chances of ovarian hyperstimulation, but in some studies at the cost of reduced pregnancy rates (Hull, 1991) and an extended period of stimulation.

The half-life of exogenous urinary FSH in females has been reported to be approximately 44 hours (Diczfalusy *et al.*, 1988; Mannaerts *et al.*, 1993), and if equal daily doses are administered, steady state serum FSH concentrations are reached after 5 to 7 days (Mizunuma *et al.*, 1990; Schoot *et al.*, 1994). It should therefore be considered that during step-up regimens elevated serum FSH concentrations may occur during the late follicular phase which may interfere with selection of a single dominant follicle (Fauser *et al.*, 1993b; Fauser, 1994; van der Meer *et al.*, 1994). It has been shown that the magnitude of FSH accumulation determines

ovarian (hyper) response (Ben Rafael *et al.*, 1986). In contrast, the dominant follicle shows a decreased dependency of FSH in the normal menstrual cycle (Messinis *et al.*, 1990; Hall *et al.*, 1991). Moreover, reduced FSH concentrations during the late follicular phase were found to be essential for monofollicular development in the monkey model (Zeleznik *et al.*, 1985). Low-dose step-up regimens ignore the concept that the FSH-threshold should be surpassed for a limited period of time only (a narrow 'window') sufficient to allow just one single follicle to be selected to gain dominance (Brown, 1978; Fauser *et al.*, 1997a). Since complications during gonadotropin induction of ovulation, such as ovarian hyperstimulation and multiple pregnancy, are related to multiple follicle development (Blankstein *et al.*, 1987), it may be worthwhile to focus more on various dose regimens and resulting patterns in FSH serum concentrations. In some ovulation induction studies (Dale *et al.*, 1993) late follicular steady-state FSH concentrations were observed during low-dose step-up regimens. However, preliminary data suggest that decreasing serum FSH concentrations may occur due to negative estrogen feedback action in some patients (White *et al.*, 1995) or using intravenous low-dose step-up FSH administration (van der Meer *et al.*, 1996).

Our group has focused on a way to establish serum FSH concentrations that mimic physiology more closely during gonadotropin induction of ovulation (Fauser, 1994). To accomplish this, a decreasing – 'step-down' – dose regimen has been developed. Initially, a starting dose of 3 ampules/day was administered in patients pretreated with GnRH-agonists. Patients were randomized for 2 different dose regimens: (1) a single fixed step-down of 1 ampule/day on day 3 followed by a fixed dose of 2 ampules/day until ovulation, or (2) a single fixed step-down of 1 ampule/day on day 3 followed by a second step-down to a daily dose of 1 ampule/day on the first day a follicle >9 mm was recognized sonographically. This treatment regimen still resulted in considerable ovarian hyper-response especially concerning medium-sized follicles and high pre-ovulatory estrogen concentrations (Schoot *et al.*, 1992b). Modification to a low-dose step-down regimen (Schoot *et al.*, 1995) with a starting dose of 2 ampules/day and each step-down by ½ ampule/day resulted in a 2.1-fold increase of serum FSH followed by a subsequent decrease of 10% per day during 4 days, remarkably similar to previous studies in the monkey model (Zeleznik *et al.*, 1986). These early studies have provided evidence that, indeed, a step-down dose regimen closely resembles late follicular phase serum FSH concentrations of the spontaneous cycle (Schoot *et al.*, 1994; van Dessel *et al.*, 1995) and can be applied in clinical practice as a safe and effective treatment alternative (van Santbrink *et al.*, 1995b). The modified step-down regimen presently used has a fixed starting dose of 2 ampules of

gonadotropin per day and from the first day a dominant follicle (a follicle ≥10 mm in diameter) is sonographically recognized, the daily gonadotropin dose is decreased by ½ ampule. Every 3 days another step down by ½ ampule is performed until the minimal daily dose of 1 ampule/day has been reached or until hCG can be administered. We have abandoned the use of GnRH agonists without any loss of clinical efficacy or safety.

1.6 OUTLINE OF THE THESIS: STUDIES AND OBJECTIVES

The study objective of this thesis was, after strictly defining the study population, to develop a regimen for induction of ovulation that mimics physiology maximally to improve treatment results (monitored by serum hormone estimation of FSH and E_2 and transvaginal sonography of follicle development in the ovaries) compared to the currently used ovulation induction regimens.

The first objective of this study was to review current opinion on endocrine and sonographic aspects concerning the normal human menstrual cycle. The second objective was to confirm and extend knowledge of physiological aspects of dynamics in follicle development in the ovary. The third objective was on one hand to review the various ways of classification of normogonadotropic infertility in literature and transpose these different criteria on our own study population using normal endocrine and sonographic values derived from a control group, and on the other hand to determine whether sonographic parameters had any predictive value for endocrine abnormalities used for PCOS. The fourth objective was to determine whether endocrine or sonographic PCOS parameters had predictive value for ovarian response in ovulation induction. The fifth objective was to compare clinical results of step-down induction of ovulation using exogenous gonadotropins with results of low-dose step-up regimens in literature. The sixth objective was to compare a step-down dose regimen for induction of ovulation using exogenous FSH with a low-dose step-up regimen in a prospective randomized way. Conclusions drawn from the studies described in this thesis are presented in the general discussion.

2

The normal menstrual cycle

2.1 GENERAL INTRODUCTION

To understand pathological conditions involved in arrested follicle maturation and anovulation, in-depth knowledge of physiology of dominant follicle selection and dominance is mandatory. As this process is still poorly understood, development of sonographic imaging combined with hormonal assays have created new perspectives for evaluation of normal physiological conditions in which the process of recruitment and selection of a dominant follicle in the human ovary takes place. A report on follicular development in the normal menstrual cycle monitored by abdominal ultrasonography was first published by Hackeloer (Hackeloer *et al.*, 1979). Only cystic structures ≥10 mm in diameter could be recognized as follicles, which resulted in monitoring of a dominant follicle only. A first report on transvaginal sonographic monitoring of follicular growth during the normal menstrual cycle (Meldrum *et al.*, 1984), indicated a big step forward regarding improved resolution. A study from our group (Pache *et al.*, 1990) generated reliable reference data for ovarian follicle number and size (follicles could be identified from a 2 mm diameter stage), throughout the normal menstrual cycle.

The objective of the present study was to confirm and extend observations of previous studies from our group, regarding FSH regulation of follicle selection and dominance monitored by frequent transvaginal sonography and blood sampling. Better understanding of the sequence of events could have clinical implications for processes involved in anovulation (disturbed follicle growth), induction of ovulation (selection of a dominant follicle) and residual ovarian activity during contraceptive medication (especially follicle dynamics during the pill-free interval).

2.2 *DECREMENTAL FOLLICLE-STIMULATING HORMONE AND DOMINANT FOLLICLE DEVELOPMENT IN THE NORMAL MENSTRUAL CYCLE*

2.2.1 Introduction

Although the human ovary has been the subject of numerous studies, many details with regard to its function are yet to be elucidated. Cyclic follicular changes in the ovary are induced by alterations in the hypothalamo–pituitary–ovarian axis. During the luteal-follicular transition serum FSH concentrations rise (Hall *et al.*, 1992) above the 'threshold' (Brown, 1978) for follicle stimulation. Consequently, a cohort of follicles gains gonadotropin dependence and continues its development, a process referred to as 'recruitment' (Goodman *et al.*, 1983). Decremental FSH concentrations during the follicular phase of the cycle appear to be essential for selection of a single dominant follicle and atresia of the remaining cohort of follicles (Zeleznik *et al*, 1985). Although FSH concentrations decline, the dominant follicle continues its growth due to enhanced sensitivity for FSH stimulation presumably caused by upregulation by intra-ovarian factors (Hsueh *et al.*, 1986).

Previous human studies concerning cyclic ovarian function chiefly involved morphology (Chikazawa *et al.*, 1986), measurement of follicle fluid steroid concentrations (McNatty *et al.*, 1979) or primary granulosa cell cultures (Erickson *et al.*, 1979; Hillier *et al.*, 1980). A major step towards improved monitoring of the dynamics of follicle development in a noninvasive way was the introduction of pelvic sonography (Hackeloer *et al.*, 1979). Using the transvaginal route, allowing the use of high frequency probes in the close proximity of the ovaries, follicles as small as 2 mm in diameter can be quantified (Pache *et al.*, 1990). Until now, few sonographic studies have been published in which follicle growth and selection in the normal human cycle has been described in detail using transvaginal sonography (TVS). Due to major individual variability, the correlation between endocrine and sonographic parameters was hampered in our previous study of normal volunteers (Pache *et al.*, 1990). The present follow-up study in carefully selected women exhibiting normal ovarian function was undertaken to investigate the relationship between decreasing FSH concentrations and follicle development (as estimated by TVS and changes in serum E_2 concentrations). More insight into FSH regulation of follicle selection and dominance under normal conditions appears to have major clinical relevance and may lead to a better understanding of residual ovarian activity during oral contraceptive medication (Fauser *et al.*, 1993a), of arrested follicle maturation in polycystic ovaries, and of ovarian

response following exogenous gonadotropins for induction of ovulation (Fauser *et al.*, 1993b).

2.2.2 Patients and methods

Subjects and study design: This study was approved by the local Ethics Review Committee, and written informed consent was obtained from each volunteer. Sixteen regularly cycling women participated in this study. They entered this study through poster advertisement and were paid for their participation. Median age was 28 years (range, 21–34 years), median body mass index (BMI; weight divided by height squared) was 22 kg/m^2 (range, 19–25 kg/m^2). All subjects had neither received hormonal treatment for at least 3 months before this study nor had they undergone any type of abdominal surgery. Daily blood samples were obtained, starting two days before expected menses (based on the length of the previous cycle) until the day of ovulation. One more sample was collected at 6 days after ovulation. Serial TVS was performed on cycle day 1 or 2, 3 or 4, 5 or 6, and so on, until there was sonographic evidence of ovulation.

Sonographic examinations were performed by two observers using a 5.0 or 7.5 MHz. transvaginal transducer. An individual was always scanned by the same observer. The ovaries were localized in relation to the iliac vessels. Follicles appeared as echo-free round or ovoid translucent structures. The follicle number was established by scanning each ovary from the inner to the outer margin in longitudinal cross sections, as described previously (Pache *et al.*, 1990; Schoot *et al.*, 1992b). Follicle size was determined from two (longitudinal and anteroposterior) or three (longitudinal, anteroposterior, and transverse) dimensions depending on the longitudinal diameter of the follicle (< 10 mm or ≥ 10 mm). In each instance the mean of the measurements was taken to be the follicle diameter.

Hormone estimations: Daily blood samples obtained through venepuncture, were centrifuged within two hours after withdrawal. Serum was stored at $-20°C$, until assayed. Serum was assayed for immunoreactive LH and FSH, E_2 and P. Kits for measurement of LH and FSH by immunoradiometric assay (IRMA) were obtained from Medgenix (Fleurus, Belgium) as described previously (Fauser *et al.*, 1990). Progesterone was estimated by RIA as described previously (de Jong *et al.*, 1974) and E_2-concentrations were estimated using RIA kits provided by Diagnostic Products Corporation (Los Angeles, CA). Intra-assay and interassay coefficients of variation were $< 5\%$ and $< 15\%$ for LH, $< 3\%$ and $< 8\%$ for FSH, $< 16\%$ and $< 17\%$ for P, and $< 15\%$ and $< 18\%$ for E_2, respectively, as described before (Pache *et al.*, 1990; Fauser *et al.*, 1990). The

applied FSH-IRMA has demonstrated a fairly good correlation with FSH bio-activity (as estimated by the *in vitro* rat granulosa cell aromatase assay) under various clinical conditions such as oligospermia (Fauser *et al.*, 1990) or anovulation (Fauser *et al.*, 1991).

Data analysis: Results are presented as the mean ± SD or median and range as indicated. Dependent on the particular evaluation, the time-scale used was based on: [1] the first menstrual day, [2] the first day of sonographic detection of a dominant follicle, or [3] the day of the LH-surge. Correlation coefficients given were Pearson's. Comparisons of various parameters within subjects were performed using Wilcoxon's test. *P* values given are two-sided, with 0.05 taken as the limit for statistical significance. Piece-wise linear regression was used to asses the relation between log(E_2) and cycle day. With this method, often referred to as the 'broken stick method' (Neter *et al.*, 1974), an estimation is made of the cycle day at which the best fitting linear relation changes to one with a greater slope. Based on previous sonographic studies the dominant follicle was defined as exceeding a diameter of 9 mm and being at least 2 mm larger than the other follicles (Pache *et al.*, 1990). The follicle also has to fit optically in the growth curve of the dominant follicle, to exclude incidental presumably atretic follicles that also fit the criteria for dominance without subsequent growth.

2.2.3 Results

Hormones: Median cycle length was 28 days (range, 26 to 31 days) with a median follicular phase of 17 days (range, 12 to 20 days) and a luteal phase of 12 days (range, 10 to 14 days). Hormone profiles (Table 1) confirmed the presence of normal ovulatory cycles in each volunteer with a median midluteal plasma P concentration of 18 ng/mL (range, 5 to 25 ng/mL [conversion factor to SI unit, 3.180]) 6 days after the LH-peak. The LH-peak occurred on cycle day 16 (range, cycle day 10–19), the median maximal plasma LH concentration was 33 mIU/mL (range, 14 to 59 mIU/mL [conversion factor to SI unit, 1.000]). The median maximal plasma FSH concentration in the early follicular phase was 6.6 mIU/mL (range, 4.4 to 11.2 mIU/mL [conversion factor to SI unit, 1.000]) on cycle day 5 (range, cycle day 1 to 9). No correlation was found between this maximal FSH concentration and (for values see Table 1) magnitude of the FSH decrease ($r = -0.37$; $P = 0.17$), duration of the follicular phase ($r = 0.14$; $P = 0.61$) or maximal preovulatory E_2 plasma concentration ($r = -0.05$; $P = 0.86$). The median minimal FSH plasma concentration in the late follicular phase (Figure 4) was found on cycle day 13 (range, cycle day 8 to 16) and was 2.9 mIU/mL (range 0.7 to 5.7 mIU/mL). The mean

Table 1 Endocrine and sonographic follicular phase characteristics of 16 participating regularly menstruating female volunteers.

	Median	*Range*
Maximum EFP* FSH conc. (IU/L)	6.6	4.4–11.2
Minimum LFP* FSH conc. (IU/L)	2.9	0.7–5.7
Foll. phase FSH decrease (IU/L/day)*	0.5	0.1–0.9
Dominant follicle:		
first sonographic appearance (CD†)	9	5–12
follicle size on CD 9 (mm)	11	9–15
growth rate (mm/day)	1.7	0.9–2.6
pre-ovulatory size (mm)	21	18–30
First day of sign. E_2 rise (CD)‡	8	4–15
Maximum LFP E_2 conc. (pg/mL)§	255	181–471
Mid-luteal P (nmol/L)	57	17–78

EFP = early follicular phase, LFP = late follicular phase.
*From cycle day 5-13.
†CD = cycle day.
‡Conversion factor to SI unit, 3.671.
§On day LH + 6

FSH-decrease during the period between maximal and minimal levels of FSH was linear ($P<0.001$) and 0.5 ± 0.05 mIU/mL per day (range, 0.1 to 0.9 mIU/mL/day), and significantly correlated ($r=0.62$; $P<0.01$; Figure 5) with the log(E_2) slope for the period between selection day and the preovulatory E_2 peak. Early follicular phase plasma E_2 concentrations for individual subjects appeared constant or increased slightly up to a certain cycle day and increased log-linearly at a higher rate thereafter. The median cycle day at which this change occurred, assessed using the broken stick method (Figure 6), was day 8 (range, day 4 to 15), 7 days before the LH-surge (range, 4 to 9 days), with a plasma E_2 concentration of 47 pg/mL (range, 25 to 97 pg/mL [conversion factor to SI unit, 3.671]). The pre-ovulatory maximal E_2 concentration (255 pg/mL; range, 181 to 471 pg/mL) was found on cycle day 15 (range, cycle day 9 to 19). The mean E_2 doubling time was 2.7 days (range, 1.8 to 3.8 days). The day of the maximal serum E_2 concentration coincided with the start of the LH-surge for each subject.

Sonography: All volunteers showed normal follicular development throughout the follicular phase, with one dominant follicle that ovulated. No significant differences were found in the distribution of the mean follicle sizes between both ovaries on cycle day one. In the early follicular phase

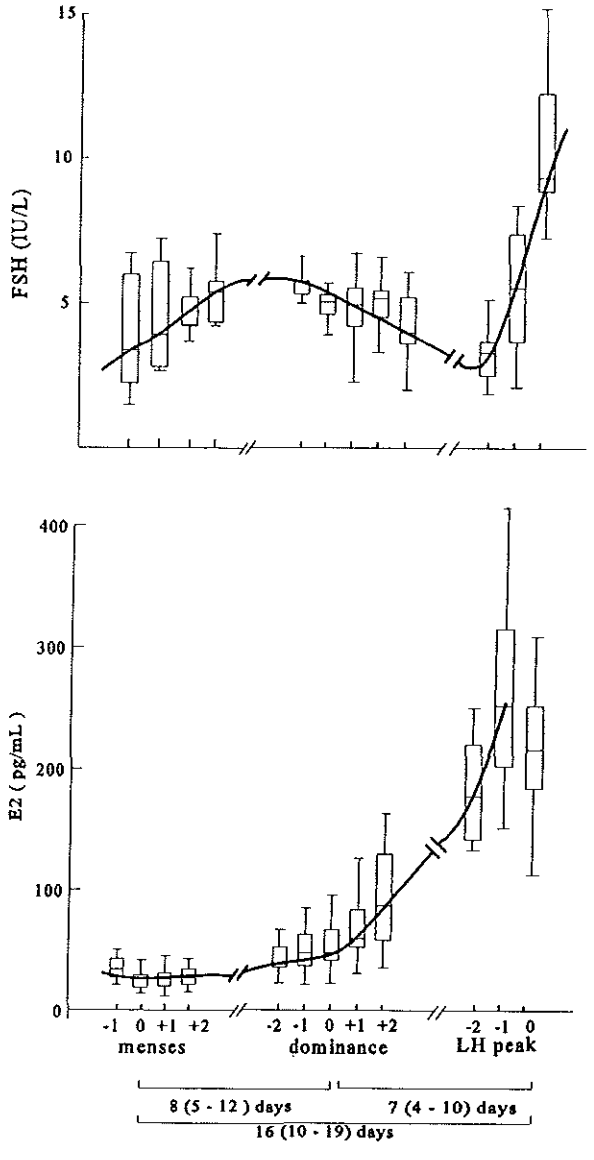

Figure 4 Box and whisker plots representing serum FSH (upper panel) and E_2 (lower panel) concentrations in 16 regularly menstruating female volunteers, synchronized around the initiation of menses, around the first day of visualization of the dominant follicle (dominance) using TVS, and preceding the serum LH peak (conversion factor to SI unit for E_2, 3.671). Boxes encompass values between the 25th and 75th percentile, horizontal lines represent median values, and whiskers give the 95% range of the values.

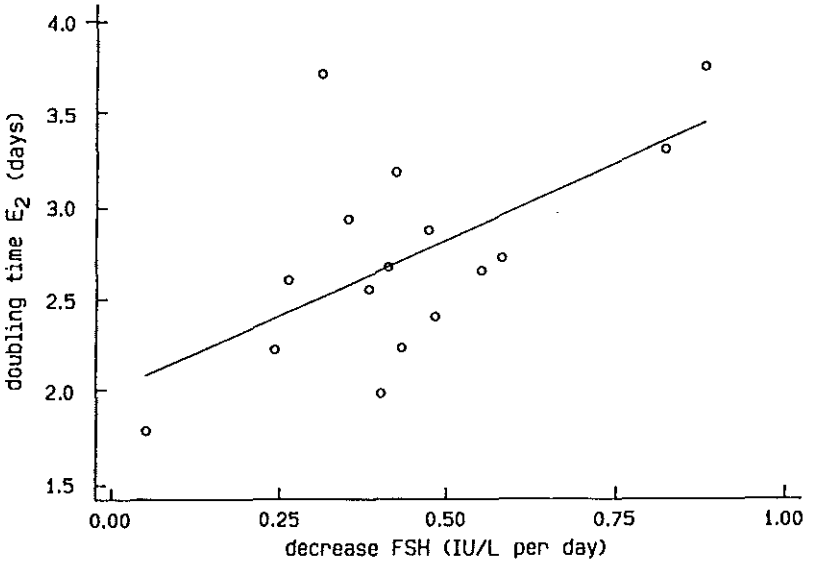

Figure 5 Correlation between the individual daily decrease in serum FSH levels versus the E_2 doubling time between cycle day 5 and 15 in 16 regularly cycling female volunteers (r = 0.6; P = 0.01).

the mean rate of follicular growth was 0.31 mm/day in the dominant ovary and 0.14 mm/day in the nondominant ovary. For the late follicular phase these slopes were −0.21 and −0.12 mm/day, respectively (data not shown). No significant difference between growth rates of nondominant follicles in the dominant and nondominant ovary in the early or late follicular phase was detected ($P = 0.22$ and $P = 0.20$, respectively). For both ovaries the mean growth slopes were significantly greater than zero ($P<0.02$) in the early follicular phase and significantly less than zero ($P<0.02$) in the late follicular phase. The first day the dominant follicle was observed by ultrasound (US) was cycle day 9 (range, cycle day 5 to 12), 6 days before the LH-surge (range, 4 to 10 days), at a diameter of 11 mm (range, 9 to 15 mm). In the nondominant ovary a follicle exceeded 9 mm in diameter and was at least 2 mm larger than the other follicles in 2 individuals. Because there was no progression in growth (no 'optically fitting in the growth curve'), these follicles were not considered to be dominant. The median growth rate of the dominant follicle was 1.7 mm/day (range, 0.9 to 2.6 mm/day), and appeared to be linear for each patient. Ovulation was detected by US on cycle day 17 (range, cycle day 13 to 20). The mean preovulatory follicle size was 21 mm (range, 18 to 30 mm).

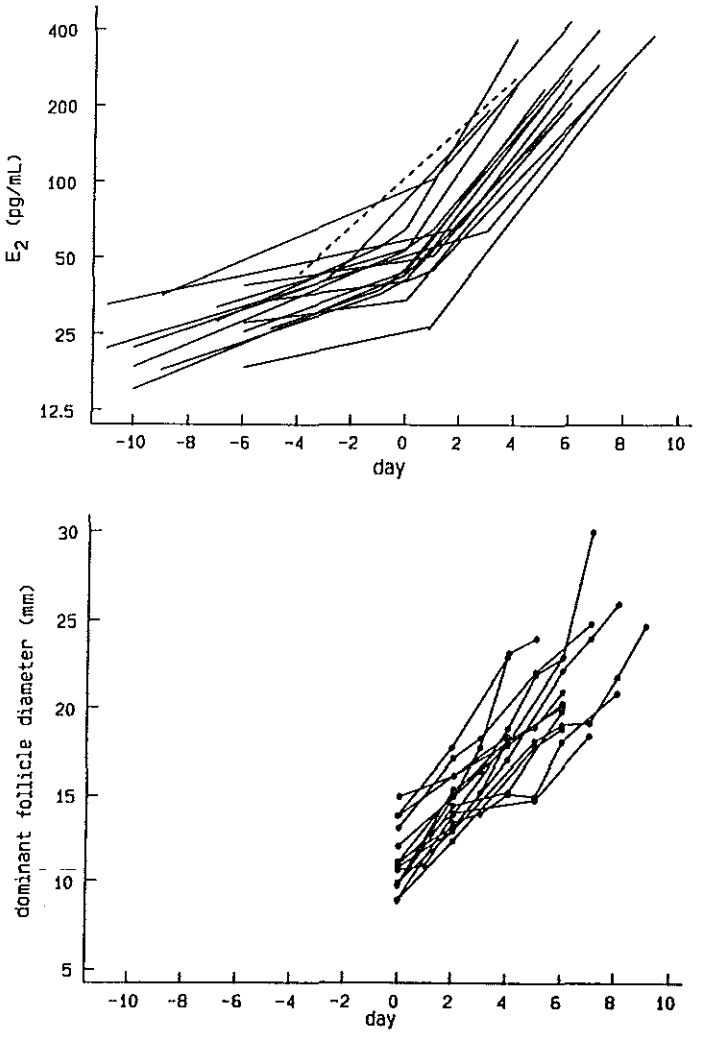

Figure 6 Rise in individual log(E_2) serum concentrations (processed with the 'broken stick' method; upper panel) and diameter of the dominant follicle (lower panel) in 16 regularly cycling female volunteers, synchronized around the first day of visualization of the dominant follicle by TVS. The dashed line in the upper panel represents one subject in which no change in E_2 slope could be established, because a dominant follicle was observed the first day of sonographic examination. Conversion factor to SI unit for E_2, 3.671.

Hormones and sonography: At the time of the early follicular FSH peak, mean duration of the period until selection of the dominant follicle was 4 days (range, 0 to 8 days). Early follicular phase maximal FSH concentrations and day of follicle selection, duration of the follicular phase, growth slope of follicles, pre-ovulatory follicle size or maximal E_2 concentrations were not correlated (data not shown). No correlation was found between the decrease of FSH plasma concentrations and increase of the diameter of the dominant follicle ($r = 0.23$; $P = 0.39$). Similarly, no correlation could be demonstrated between decrease of FSH concentrations (or duration of this decrease) and day of selection of the dominant follicle, duration of the follicular phase, preovulatory dominant follicle size or maximal preovulatory E_2 concentrations (data not shown). The first day of a significant rise in E_2 plasma concentration correlated strongly with the day at which the dominant follicle appeared sonographically ($r = 0.84$; $P < 0.001$; Figure 6). From the day the dominant follicle was first seen by TVS (median cycle day 8) until the day of the maximal E_2 plasma concentration (median cycle day 15), there was a significant correlation between the rate of increase in E_2 plasma concentrations and dominant follicle growth ($r = 0.53$; $P = 0.04$).

2.2.4 Discussion

In this longitudinal study an attempt was made to correlate decremental serum FSH concentrations during the follicular phase of the normal menstrual cycle with dominant and nondominant follicle development. Frequent TVS and measurement of E_2 serum concentrations were used to monitor follicle development. All cycles in this study appeared ovulatory, with gonadotropin and steroid hormone concentrations within the normal range according to other studies (Hackeloer *et al.*, 1979; Landgren *et al.*, 1980).

In the early follicular phase 2.5-fold differences in maximum FSH concentrations were observed. Moreover, correlations between maximum FSH concentrations and follicular phase characteristics (such as follicular phase length or maximum E_2 concentrations) were absent suggesting that distinct differences in FSH threshold concentrations for ovarian stimulation are present even in this carefully selected group of young, healthy, normal-weight women exhibiting normal ovarian function. This observation supports and extends the ovarian 'threshold concept', initially put forward by Brown (Brown *et al.*, 1978), suggesting that every woman has an individual FSH concentration that should be surpassed for stimulation of development of a cohort of follicles. Under normal conditions, the rise in FSH takes place during the luteal-follicular transition and is correlated closely with the preceding LH peak as opposed to menses (Hall *et al.*,

1992). Therefore, it should be preferred to use the previous LH peak rather than cycle day (which refers to the initiation of menses) as point of reference to characterize regulation of follicle growth. A rise in serum FSH and subsequent follicle growth can also be induced artificially by removal of the corpus luteum or the dominant follicle (Nilsson *et al.*, 1982), indicating that follicles that are ready to be recruited are present throughout the cycle. Maximum FSH concentrations of 6.6 mIU/mL (range, 4.4 to 11.2 mIU/mL) were reached on cycle day 5, followed by a linear decrease of 0.5 mIU/mL per day (range, 0.1 to 0.9 mIU/mL per day) until cycle day 13 (3 days before the LH peak). It appears that the magnitude of decrease of endogenous FSH concentrations is independent of initial FSH concentrations. During the period between cycle day 5 and 15 the slopes for FSH and E_2 concentrations showed a significant negative correlation, indicating that late follicular phase E_2 production (presumably by the dominant follicle) may be stimulated to a greater extent in women with a slower decrease in FSH. Hence, the magnitude of stimulation of the aromatase enzyme (and subsequent estrogen biosynthesis) by the dominant follicle is related closely to the extent of FSH stimulation. The dominant follicle becomes less dependent on FSH stimulation, allowing continuation of its development in the late follicular phase, whereas remaining follicles of the same cohort undergo atresia due to insufficient stimulation by FSH. The association between FSH and E_2 concentrations was studied previously in the monkey model, which led the authors to conclude that FSH secretion is suppressed by E_2 to accomplish selection of a single dominant follicle. Indeed, interference with decremental FSH concentrations (using E_2 antibodies) resulted in multiple follicle development (Zeleznik *et al.*, 1985). Increasing concentrations of inhibin during the follicular phase also have been shown to inhibit pituitary FSH release (Burger, 1993).

Aromatase activity was found to be present in granulosa cells obtained from follicles beyond 6 to 8 mm in diameter (Erickson *et al.*, 1979; Hillier *et al.*, 1980), and a close correlation between follicle fluid E_2 concentrations and follicle diameter has been established (McNatty *et al.*, 1979). In our study a clear rise in plasma E_2 was seen from cycle day 8 onwards (coinciding with the sonographic visualization of a dominant follicle at a size of >9 mm). This indicates that the induction of aromatase activity in the follicle destined to gain dominance does not contribute to serum E_2 concentrations before the follicle can be discerned as the dominant one. The observation that endocrine and sonographic indications of dominant follicle selection exhibit a close resemblance, strongly indicate that visualization of the dominant follicle by TVS represents a relevant biological phenomenon. Our sonographic data on the development of the dominant follicle match quite well with previous studies. A linear growth rate of

1.7 mm/day was found for the dominant follicle with a median preovulatory follicle size of 21 mm (Hackeloer *et al.*, 1979; Pache *et al.*, 1990; Eissa *et al.*, 1986). There is some controversy with regard to a correlation between dominant follicle diameter and serum E_2 concentrations in previous studies using abdominal sonography (Lenz, 1985). Using morphology, however, a clear correlation has been established (Chikazawa *et al.*, 1986). In the present study a significant correlation between increase of serum E_2 concentrations and dominant follicle growth was found in support of the notion (Hackeloer *et al.*, 1979; Baird *et al.*, 1975) that circulating E_2 is produced mainly by the dominant follicle. The absence of a correlation between decreasing FSH and follicle size indicates that growth of the dominant follicle may be dependent on other (intraovarian) factors next to FSH.

In conclusion, the present study in female volunteers with normal menstrual cycles indicates that a major difference in FSH threshold concentrations for stimulation of follicle development does exist. It was confirmed that the dominant follicle exhibits enhanced sensitivity for FSH and the magnitude of follicular phase decrease in FSH affects dominant follicle E_2 production. In addition, appearance of the dominant follicle on US is related closely to a distinct rise in serum E_2 concentrations. These notions may have major clinical implications for various conditions such as induction of ovulation using exogenous gonadotropins or residual ovarian activity during steroid contraceptive medication. Interestingly, enhanced sensitivity of the dominant follicle for FSH has been demonstrated also in clomiphene-citrate resistant anovulatory PCOS patients during exogenous gonadotropins administered in decremental doses (Schoot *et al.*, 1993). The resemblance with the pattern of endogenous FSH concentrations reported here is striking, which emphasizes the need for a further evaluation of the concept of elevated FSH threshold in PCOS patients. Moreover, at the end of the seven-day pill-free interval during oral contraceptive medication, serum FSH concentrations are in the same order of magnitude as during the early follicular phase of normal cycles. It therefore seems reasonable to assume that follicle recruitment already has been initiated before contraceptive medication of the next month is started. Indeed, ovarian follicles exceeding 10 mm in diameter have been observed in approximately 30% of women using oral contraceptives (for review see Fauser *et al.*, 1993b). Apparently, follicle maturation is arrested at a stage just before dominant follicle selection (and the diminished need for FSH). Altogether, data presented in this study may serve as a reference for better understanding of the above mentioned conditions, and may elicit further studies with regard to mechanisms regulating normal and abnormal follicle development in the human.

3

Polycystic ovary syndrome

3.1 GENERAL INTRODUCTION

PCOS is a poorly understood and poorly defined heterogeneous entity. The fact that several etiological factors lead to a common endpoint of polycystic changes in the ovary, may contribute to confusion. Parameters used for PCOS diagnosis in patients presenting with anovulatory infertility include elevated serum LH concentrations, hyperandrogenemia, or polycystic ovaries on ultrasound. The fact that diagnostic criteria used in the literature are not identical may have added to confusion and have weakened the development of effective and safe therapeutic options. This is of importance, since treatment of infertility associated with PCOS may be difficult, and response to medication is accompanied by high rates of hyperstimulation and early pregnancy loss (Regan *et al.*, 1990).

In the following study we have attempted to evaluate various patient characteristics used in the literature for PCOS diagnosis: first we set strict normal cut-off levels for these criteria in a control population and transposed these to the abnormal population using anovulatory normogonadotropic infertility as inclusion criterion. In a second preliminary study we compared various patient characteristics as markers of disease and tried to validate their usefulness as a predictor of treatment response in ovulation induction using exogenous gonadotropins.

3.2 *CLASSIFICATION OF NORMOGONADOTROPIC ANOVULATORY INFERTILITY: POLYCYSTIC OVARIES DIAGNOSED BY ULTRASOUND VERSUS ENDOCRINE CHARACTERISTICS OF POLYCYSTIC OVARY SYNDROME*

3.2.1 Introduction

Polycystic ovary syndrome (PCOS) represents a heterogeneous group of patients with a variety of underlying abnormalities. Moreover, the lack of uniformity in criteria used for PCOS diagnosis further adds to confusion surrounding this syndrome. Initially, it was reported by Stein and Leventhal that morphology diagnosis of polycystic ovaries was associated with a

specific medical history and characteristic findings upon physical examination (infertility, oligo/amenorrhea and masculinization). More recently, diagnosis of PCOS was based on clinical symptoms and biochemical parameters. At present, some investigators apply just a single biochemical marker such as elevated serum LH concentrations (Scheele *et al.*, 1993) or high androgen concentrations (Lobo, 1985) to define PCOS, whereas others use a combination of these two parameters (Ardaens *et al.*, 1991).

As pelvic ultrasound (US) was introduced in the early 1980s, this new and noninvasive technique allowed morphological assessment of the ovaries on a wide scale. Sonographic appearance of polycystic ovaries became an important criterion for PCOS diagnosis (Adams *et al.*, 1985; Balen *et al.*, 1995; Fox *et al.*, 1991). In fact, many investigators now use US as the sole criterion for diagnosis of this syndrome (Balen *et al.*, 1995; Fox *et al.*, 1991; Takahashi *et al.*, 1993). Sonographically diagnosed polycystic ovaries can be enlarged (Puzigaca *et al.*, 1991; Pache *et al.*, 1993), contain an increased number of follicles (Adams *et al.*, 1985; Obhrai *et al.*, 1990), or exhibit an increased amount or density of stroma (Adams *et al.*, 1985; Dewailly *et al.*, 1994). These characteristics are sometimes used as a single parameter or are combined (Adams *et al.*, 1985; Balen *et al.*, 1995, Dewailly *et al.*, 1994). At present, transvaginal sonography (TVS) with enhanced resolution is most commonly used for US evaluation of ovaries. However, criteria determined by transabdominal US (Adams *et al.*, 1985) are still used frequently (Balen *et al.*, 1995; Obhrai *et al.*, 1990; Robinson *et al.*, 1992).

Over 10 years after the introduction of pelvic US, still no agreement has been reached on criteria used for polycystic ovary diagnosis nor on its validity. It is unclear to what extent the presence of polycystic ovaries overlaps with other endocrine criteria used to diagnose PCOS such as elevated LH or androgen concentrations. It appears essential to evaluate various criteria for PCOS diagnosis to create a stronger basis for further uniform evaluation of this entity.

3.2.2 Patients and methods

This study was approved by the local Ethics Review Committee and informed consent was obtained from all participants. Forty-eight control subjects were selected by poster advertisement and paid for participation. Inclusion criteria were regular menstrual cycle (26 to 30 days), age between 20 and 35 years, normal weight (body mass index [BMI; weight divided by height squared] : 18–26 kg/m^2), and no hormone therapy for the last 3 months. On cycle day 3, 4 or 5, TVS was performed and a blood sample was taken. For sonographic imaging, we used a 6.5 MHz trans-

vaginal transducer (Model EUB-415; Hitachi Medical Corporation, Tokyo, Japan). The ovaries were localized in relation to the iliac vessels. Follicles appeared as round or ovoid translucent structures. The follicle number was established by scanning each ovary from the inner to the outer margin in longitudinal cross-sections (Pache *et al.*, 1991). The ovarian volume was estimated according to the following formula: ½(AxBxC), where A is the longitudinal diameter, B the anteroposterior diameter, and C the transverse diameter of the ovary (Sample *et al.*, 1977). Mean follicle number and mean ovarian volume were calculated as the addition of left and right divided by two. Ovarian stroma echogenicity was scored as 1 (normal), 2 (moderately increased) or 3 (markedly increased) as described by Pache (Pache *et al.*, 1991). Total stroma count was the stroma score addition of the left and the right ovary.

Three hundred and fifty patients attending our Fertility Clinic between 1991 and 1994 with [1] infertility, [2] oligomenorrhea (interval between periods >35 days) or amenorrhea (absence of vaginal bleeding for at least 6 months), and [3] serum FSH concentrations within normal limits (1 to 10 mIU/mL [conversion factor to SI unit, 1.000]; van Santbrink *et al.*, 1995a) were included in this study. TVS and blood withdrawal were performed at random. PRL concentrations were not used as an exclusion criterion because hyperprolactinemia has been described both in hypogonadotropic as well as in PCOS patients. Timing of sampling may in some cases affect the magnitude of serum LH concentrations (due to presence of previous exposure to negative feedback action of high P concentrations or due to midcycle surge LH concentrations in oligomenorrheic ovulatory patients). All patients with periovulatory E_2 concentrations (E_2 >109 pg/mL [conversion factor to SI unit, 3.671]) or luteal P concentrations (P >4.7 ng/mL [conversion factor to SI unit, 3.180) were excluded ($n = 8$ and $n = 12$, respectively). This resulted in a total study population of 330 patients.

Hormone estimations: Blood samples were obtained through venepuncture and centrifuged within two hours after withdrawal. Serum was stored at −20°C and assayed for FSH and LH by immunoradiometric assay (IRMA) kits provided by Medgenix (Fleurus, Belgium), and AD and T by radioimmunoassay (RIA) kits provided by Diagnostic Products Corporation (Los Angeles, CA), as described previously (Fauser *et al.*, 1991). Intra-assay and interassay coefficients of variation were less than 3% and 8% for FSH, less than 5% and 15% for LH, less than 8% and 11% for AD and less than 3% and 5% for T, respectively.

Data analysis: Results are presented as the mean ± SD if normally distributed, or median and range if distributed otherwise. Normal distribution of

sonographic parameters was tested with the Kolmogorov–Smirnov goodness of fit test. Normal range was computed according to a p5 to p95 confidence interval according to mean ± 1.645 x SD if normally distributed (mean ovarian volume, mean follicle number and LH concentration), or it was antilogged before calculation if parameters were not normally distributed (AD and T concentrations). We compared groups using the Mann–Whitney and Wilcoxon rank-sum test and the Chi-square analysis of contingency table.

Used criteria for PCOS diagnosis included [1] elevated LH concentrations, [2] elevated serum androgen concentrations (increased AD and/or T concentrations), and [3] polycystic ovaries on TVS (defined as increased mean ovarian volume and/or mean follicle number). The upper limit of normal for sonographic and endocrine parameters was defined based on the 95th percentile of observations in the control population.

3.2.3 Results

Control subjects ($n = 48$) were used to determine the normal range for sonographic and endocrine parameters. Median age was 27 years (range, 21 to 35 years), cycle length was 28 days (range, 27 to 30 days) and BMI was 22 kg/m^2 (range, 18 to 26 kg/m^2). Follicle number per ovary and ovarian volume were distributed normally. Mean number of follicles per ovary was 7.0 ± 1.7 (upper limit: 9 follicles), mean ovarian volume was 6.8 ± 2.4 mL (upper limit: 10.7 mL), and total stroma count for both ovaries never exceeded 3 (Figure 7). Mean LH serum concentration was 4.1 ± 1.8 mIU/mL (upper limit 6.9 mIU/mL, conversion factor to SI unit, 1.0), median AD concentration was 2.48 ng/mL (range, 1.14 to 5.29 ng/mL, upper limit 4.41 ng/mL; conversion factor to SI unit, 3.671) and median T concentration was 0.58 ng/dL (range, 0.29 to 1.12 ng/dL, upper limit 0.89 ng/dL; conversion factor to SI unit 3.467).

The study population of 330 normogonadotropic oligomenorrheic or amenorrheic women with infertility had a median age of 28 years (range, 17 to 41 years), BMI was 25 kg/m^2 (range, 18 to 55 kg/m^2) and median duration of infertility was 2 years (range, 1 to 13 years). Serum prolactin concentrations were elevated in 28 subjects (8%), in this subgroup correlations between US and endocrine parameters were not different from the overall population (data not shown). Age, BMI and duration of infertility were distributed equally in both groups (data not shown). Distribution of all sonographic criteria (increased follicle number, ovarian volume, and stroma count based on the upper limit of normal defined in control subjects) involved in polycystic ovary diagnosis in the total study group are depicted in Figure 8 (*top*). Increased ovarian volume (41% of total group) showed 90% overlap

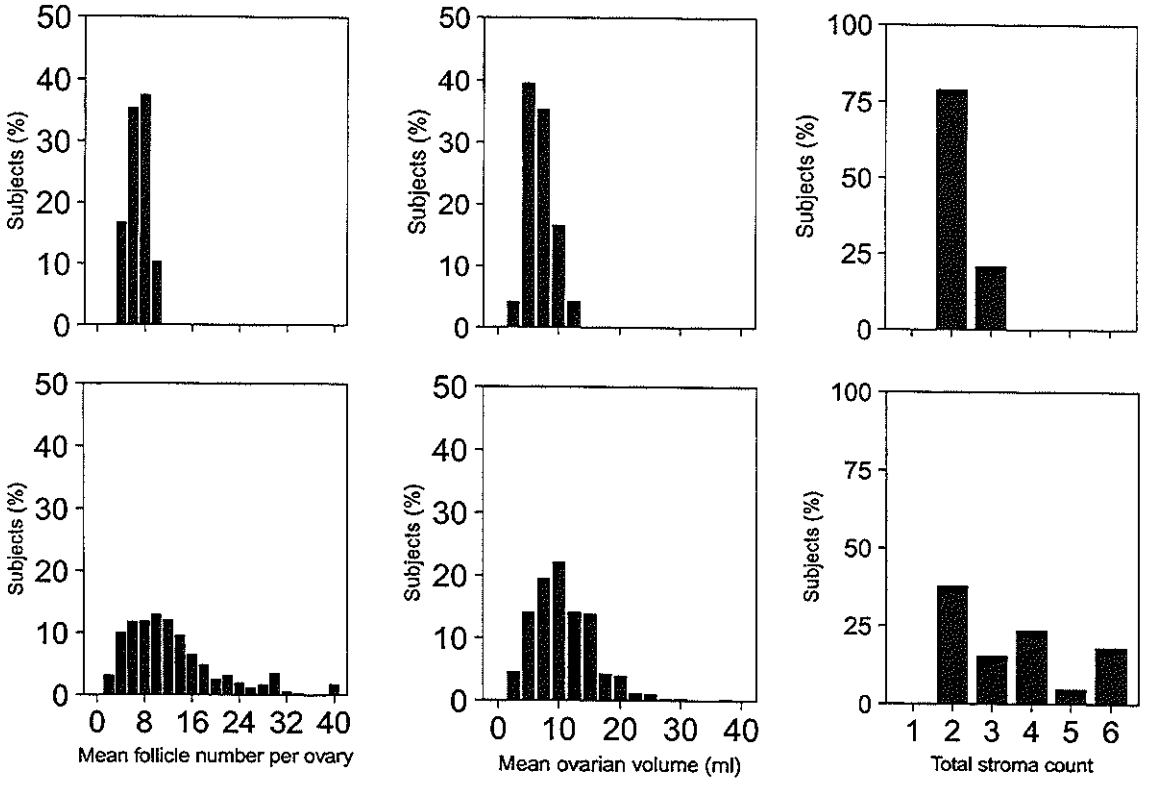

Figure 7 Distribution of mean follicle number per ovary, mean ovarian volume and total stroma count (both ovaries) in regularly cycling controls (n = 48; upper panel) and normogonadotropic oligomenorrhoea or amenorrheic infertile patients (n = 330; lower panel).

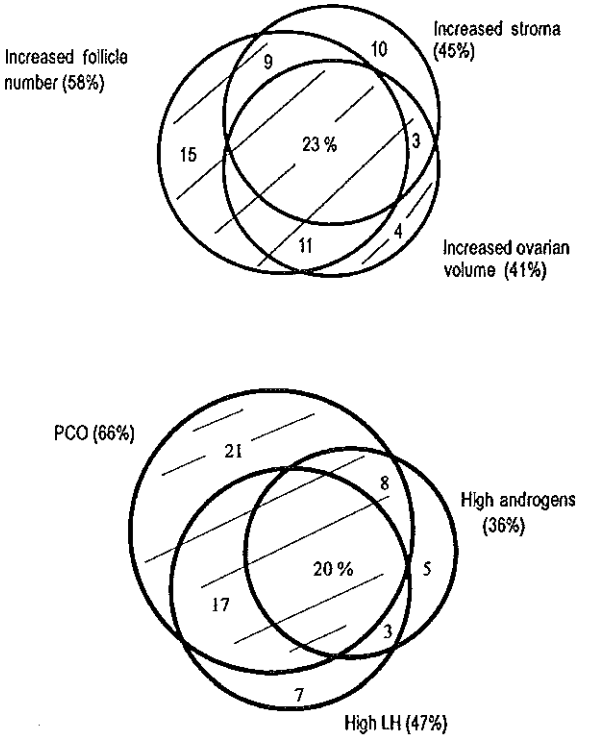

Figure 8 Distribution of sonographic characteristics (increased mean number of follicles [≥10 per ovary], increased mean ovarian volume [≥10.8 mL] and increased total stroma count for both ovaries [>3] (upper panel), and PCOS diagnostic criteria (elevated androgens [AD ≥4.44 ng/mL {conversion factor to SI unit, 3.671} and/or T ≥0.92 ng/dL {conversion factor to SI unit, 3.467}], elevated LH [LH ≥7.0 mIU/mL, (conversion factor to SI unit, 1.0)] and polycystic ovaries [mean ovarian volume ≥10.8 mL and/or mean number of follicles per ovary ≥10) (lower panel) in 330 normogonadotropic oligomenorrhoea or amenorrheic infertile patients. Numbers given are percentages of the overall study group. The hatched area in both figures represent an identical patient group, characterized by polycystic ovaries.

with increased follicle number (58% of total group) and/or increased total stroma count (45% of total group). For increased follicle number or increased total stroma count, overlap with both remaining US parameters was 74% and 78%, respectively. In the total study population, 217 (66%) patients showed polycystic ovaries on sonographic examination (arbitrarily defined as mean ovarian volume ≥10.8 mL and/or mean follicle number per ovary ≥10). One hundred and twenty (36%) patients exhibited elevated serum androgens (AD ≥4.44 ng/mL and/or T ≥0.92 ng/dL), and 155 (47%) patients

showed increased serum LH concentrations (LH ≥ 7.0 mIU/mL). Sonographic and/or endocrine criteria were not increased in 62 (19%) patients of the study group. Three groups with abnormal endocrine or sonographic parameters had extensive overlap as can be seen in a Venn-diagram showing the distribution of percentages of patients in the various groups (Figure 8, *bottom*). Sixty-eight (21%) patients presented with elevated free-androgen index (FAI: T x 100/ sex-hormone binding globulin ratio >9.1). Correlations with US parameters were not different from T (data not shown).

In the total study population 252 (76%) patients were oligomenorrheic and 78 (24%) patients were amenorrheic. Amenorrheic patients had significantly higher serum T concentrations ($P = 0.02$), mean follicle number ($P = 0.04$), and total stroma count ($P < 0.001$) (data not shown). Luteinizing hormone serum concentrations of oligomenorrheic and amenorrheic patients were not significantly different (data not shown). There was no major difference when Venn-diagrams were plotted for oligomenorrheic and amenorrheic patients separately (data not shown).

Sensitivity and specificity of various sonographic parameters (mean follicle number per whole ovary, mean ovarian volume and total stroma count) was calculated for elevated T, AD, and LH concentrations (Table 2). The best sonographic test for prediction of hyperandrogenicity was increased mean ovarian volume, with a sensitivity of 57% and a specificity of 67%. The addition of increased stroma score to mean ovarian volume and follicle number did not increase the predictive value of the tests (data not shown). Receiver operating characteristics (ROC) curves of polycystic ovaries (increased ovarian volume and/or follicle number) as a test parameter and endocrine PCOS criteria (elevated LH or T concentrations) showed limited predictive value of sonography for abnormal endocrine PCOS criteria (Figure 9). The best correlations were between [1] ovarian volume and AD ($r = 0.36$; $P < 0.001$) or T ($r = 0.35$; $P < 0.001$) serum concentration, and [2] follicle number and T ($r = 0.33$; $P < 0.001$) or LH ($r = 0.30$; $P < 0.001$) concentration (data not shown).

3.2.4 Discussion

A lack of consensus on criteria used for PCOS diagnosis is recognized widely (Dunaif *et al.*, 1992). Often accurate information regarding the used definition of PCOS is insufficiently provided in the literature. Moreover, the population from which PCOS patients is obtained is poorly characterized. This may cause discrepant observations in different groups of patients – all termed PCOS – regarding prevalence of this syndrome, abnormalities present, and treatment outcome. Correlations between sonographic (ovarian volume, follicle number and stroma count) and

Table 2 Sensitivity, specificity, predictive value and significance of single and multiple sonographic criteria for polycystic ovary diagnosis versus single and multiple endocrine characteristics of PCOS.

Test parameter	*Reference test*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Total*	*P**
Increased ovarian volume (≥10.8 ml)	High androgen levels[†]	57	67	49	74	64	<0.001
	High LH levels[‡]	50	67	58	59	59	0.002
	High LH & androgens	61	65	35	84	64	<0.001
Increased follicle number (≥10 follicles)	High androgen levels	70	47	42	73	55	0.03
	High LH levels	70	50	56	65	60	<0.001
	High LH & androgens	76	46	30	87	53	0.001
Increased stroma score (≥4)	High androgen levels	52	57	40	68	55	NS
	High LH levels	54	60	56	58	57	0.015
	High LH & androgens	53	56	27	79	55	NS
Increased ovarian volume and/or follicle number	High androgen levels	77	41	43	76	54	0.001
	High LH levels	77	45	56	68	60	<0.001
	High LH & androgens	84	40	30	89	50	<0.001
Increased ovarian volume and follicle number	High androgen levels	49	73	50	72	65	<0.001
	High LH levels	42	73	59	58	58	0.005
	High LH & androgens	53	71	35	83	67	<0.001

PPV = positive predictive value.
NPV = negative predictive value.
Total = percentage correctly identified (positive and negative) of total group.

*Chi-square test for the association between test-parameter and reference test.
[†]High androgen levels = A ≥4.44 ng/mL (conversion factor to SI unit, 3.671) and/or T ≥92 ng/dL (conversion factor to SI unit, 3.467).
[‡]High LH levels = LH ≥7.0 mIU/mL (conversion factor to SI unit, 1.0).

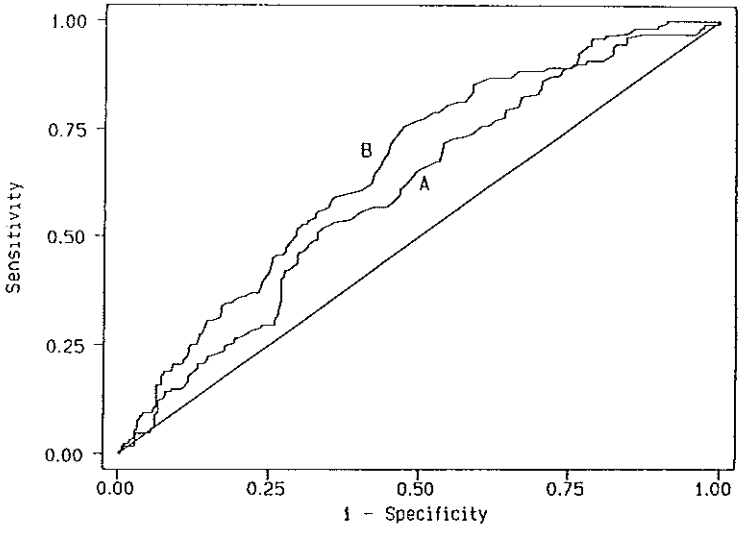

Figure 9 Receiver Operating Characteristics (ROC) curves of mean ovarian volume for elevated LH levels (≥7.0 mIU/mL, conversion factor to SI unit, 1.0) (A) and elevated T levels (≥0.92 ng/dL [conversion factor to SI unit, 3.467]) (B) in a group of 330 normogonadotropic oligomenorrhoea or amenorrheic infertile patients. The diagonal line represents an imaginary test which has no discriminative power.

endocrine (LH, AD, and T serum concentrations) characteristics in PCOS patients have been described by various authors. For instance, increased serum LH and androgen concentrations were demonstrated to be related to ovarian enlargement (Balen *et al.*, 1995; Robinson *et al.*, 1992). In a group with bilateral polycystic ovaries, on US it was shown that, in patients with a more PCO-like sonographic image (more follicles per ovary), biochemical disturbances were more apparent (Takahashi *et al.*, 1993). Correlation between serum LH concentrations, androgen concentrations, and sonographic parameters was also observed in normogonadotropic anovulatory infertility (Pache *et al.*, 1993), which is confirmed in the present study (data not shown). The predictive value of sonographic characteristics for endocrine abnormalities frequently associated with PCOS (elevated LH, AD or T concentrations) is hard to find in the literature. Fox and colleagues (Fox *et al.*, 1991) studied a group of 65 oligomenorrheic or amenorrheic women and suggested that LH and FAI were good predictors for polycystic ovaries, although LH and T concentrations as single markers had a specificity of both 60% and a negative predictive value of 46% and 49%, respectively. We found comparable sensitivity

and specificity of sonographic parameters (polycystic ovaries) for the prediction of endocrine abnormalities (see Table 2).

In the present study normal values for sonographic and endocrine parameters used for PCOS diagnosis were defined in a well-characterized control population of volunteers ($n = 48$). These women had strict regular cycles, normal weight, and were aged between 20 and 35 years. PCOS diagnostic criteria (increased follicle number and ovarian volume, elevated serum T and AD and elevated serum LH) were defined as the 95th percentile of the control population. The prevalence of polycystic ovaries in this population was 8%. Several other studies (Polson *et al.*, 1988; Farquhar *et al.*, 1994) showed that polycystic ovaries can be observed in approximately 22% of 'normal' randomly selected women. Differences in inclusion criteria for the control population ('women not seeking medical treatment' [Polson *et al.*, 1988] versus rigid criteria used in the present study) and different criteria to define polycystic ovaries easily can explain these seemingly discrepant observations. We chose increased mean ovarian volume and/or increased mean number of follicles per whole ovary as criteria for polycystic ovaries. The first criterion was chosen because: TVS has been described to be an accurate and objective way to measure ovarian volume (El Tabbakh *et al.*, 1986), and mean ovarian volume exhibits 90% overlap with the remaining 2 sonographic criteria. Mean follicle number and total stroma count show only 74% and 78% overlap (Figure 8, *top*). Mean follicle number was chosen as criterion next to mean ovarian volume because it increases the sensitivity as compared with volume alone (Table 2). Additionally, follicle number is the most frequently used and investigated sonographic parameter since its introduction as diagnostic criterion for polycystic ovaries by Adams *et al.* (Adams *et al.*, 1985). The value of stroma density in PCOS diagnosis has been questioned in recent studies, because it appears to be a subjective parameter (Ardaens *et al.*, 1991; Dewailly *et al.*, 1994). The value of sonographic parameters as a screening test to predict endocrine abnormalities characteristic for PCOS appears to be limited in the present study. Because a good screening test must have high sensitivity (i.e., percentage of the test indicates presence of the disease of the total disease group) rather than specificity, mean follicle number as a single test and mean follicle number and/or ovarian volume as a combined test appear the most favorable (Table 2). Although the combined sonographic test has the highest sensitivity, the ROC curves (Figure 9) show that the AUC for the prediction of both elevated T or LH concentrations did not exceed 0.65 and therefore may be categorized as a test with limited discriminative power.

Only normogonadotropic (WHO class II [World Health Organization, 1993]) oligomenorrheic or amenorrheic infertile patients were included in

this study. We chose this particular classification based on serum FSH concentrations within normal limits, because PCOS has been described as part of this group. Subdivision of these patients according to criteria used to identify PCOS (Fig 8, *bottom*) showed much overlap in the 3 groups of polycystic ovaries, elevated LH concentrations, and hyperandrogenemia, whereas 19% of subjects did not fit any criterion. In addition, only 20% of patients fulfilled all criteria. Recent studies claim that US has a tendency to overestimate PCOS (Abdal Gadir *et al.*, 1992). This is in line with the present results indicating that 21% of patients present with polycystic ovaries without endocrine signs of PCOS. Elevated serum LH concentration and slightly decreased FSH concentration are well-established criteria for PCOS diagnosis. This also can be expressed as the LH:FSH ratio. Although this ratio has been used as an important diagnostic criterion in some studies (Scheele *et al.*, 1993; Fox *et al.*, 1991), recent literature shows reduced emphasis on measurements of serum gonadotropin concentrations for PCOS diagnosis (Obhrai *et al.*, 1990; Lobo *et al.*, 1983). Pulsatile release of LH as well as timing of blood withdrawal and assay variability (RIA vs. IRMA) makes it questionable to use this parameter as the sole diagnostic criterion (Robinson *et al.*, 1992; Fauser *et al.*, 1992). In our study, 85% of patients presenting with elevated serum LH concentrations suffered from polycystic ovaries and/or elevated serum androgens.

Hyperandrogenicity is perceived as the most important biochemical parameter for PCOS diagnosis (Dunaif *et al.*, 1992). Steroid hormones of adrenal or ovarian origin (T, AD, and dehydroepiandrosterone sulfate [DHEAS]) are frequently elevated in PCOS patients. Dehydroepiandrosterone sulfate is a marker for adrenal hyperandrogenism (Rittmaster *et al.*, 1994), but generally is excluded as a PCOS diagnostic criterion. Most authors agree that T and AD are the best parameters for detection of hyperandrogenism (Fox *et al.*, 1991; Pache *et al.*, 1993; Robinson *et al.*, 1992). In practice, hyperandrogenicity is defined most frequently as elevated serum AD and/or T concentrations. Androstenedione concentration and ovarian enlargement have been reported to be correlated in PCOS (Balen *et al.*, 1995; Puzigaca *et al.*, 1991), which is confirmed in the present study ($r = 0.36$; $P < 0.001$). However, the most sensitive single androgen for discrimination of PCOS is reported to be T (Pache *et al.*, 1993; Robinson *et al.*, 1992), which equals present results. Serum androgen concentration may be the most valuable marker for PCOS, because this is correlated best to both sonographic observations and elevated LH concentrations.

We conclude that – after defining in a control population strict cutoff levels for sonographic and endocrine PCOS diagnostic criteria – consider-

able overlap between these parameters was found in normogonadotropic oligomenorrheic or amenorrheic infertile women. However, the predictive value of polycystic ovaries (on US) for endocrine abnormalities was limited. This cross-sectional study should be followed by a longitudinal follow-up study to investigate which of these initial screening parameters are best predictors for success or complication rates during induction of ovulation.

3.3 *PREDICTORS OF FOLLICLE DEVELOPMENT IN GONADOTROPIN INDUCTION OF OVULATION USING A DECREMENTAL DOSE REGIMEN*

3.3.1 Introduction

Although an extensive number of classifications for anovulatory infertile patients have been proposed over the years, non of these – mostly descriptive – classifications seem to correlate with treatment outcome following gonadotropin induction of ovulation. Patient characteristics like age, obesity, and duration of infertility for example are known for their influence on treatment outcome (Dor *et al.*, 1980; McCluer *et al.*, 1992). Recently it has been reported that parameters can be identified in infertile couples that predict chances for spontaneous conception and attempts are made to combine these as a predictive model for overall chances of conception (Eimers *et al.*, 1994; Collins *et al.*, 1995). In addition, it should certainly be helpful if treatment response following ovulation induction could be predicted in anovulatory patients. It is generally stated that polycystic ovary syndrome (PCOS) patients are at risk of low success rates when treated with clomiphene-citrate for induction of ovulation. Therefore the majority of PCOS patients need exogenous gonadotropins as a second-line therapy. Could it be possible to divide the overall group into gonadotropin 'good-responders' and 'bad-responders' based on initial screening? In the literature there is a lack of uniformity in defining subgroups in normogonadotropic oligo- or amenorrheic infertility (WHO II; van Santbrink *et al.*, 1997a). Of the various criteria used to describe this subgroup, apart from clinical symptoms, the most frequently reported are serum LH, serum androgens (elevated AD and/or T) and sonographic criteria for polycystic ovaries (ovarian volume and follicle number).

The objective of the present study is to evaluate criteria which have been used previously by our group for classification (clinical, endocrine, and sonographic characteristics), and to try to validate their usefulness as a predictor of treatment response in a group of infertile patients with normogonadotropic clomiphene-resistant oligo- or amenorrhea treated with exogenous gonadotropins.

3.3.2 Patients and methods

Patients: One hundred and eight patients presenting with infertility, oligomenorrhea (interval between periods ≥35 days) or amenorrhea (absence of vaginal bleeding ≥6 months) and serum FSH concentrations within normal limits (1–10 IU/L) were included in this study. All participants suffered from clomiphene-resistant anovulation (CRA, failure to ovulate despite increasing dose of clomiphene-citrate, with doses up to 150 mg/day from cycle day 3 to 7 during at least 3 consecutive cycles) or clomiphene-failure (defined as failure to conceive despite increasing dose of clomiphene-citrate, with doses up to 150 mg/day from cycle day 3 to 7 during at least 3 consecutive cycles). In our population the majority of participants was CRA and about 25% were clomiphene-failure patients. Because the study clinic is a tertiary referral center, a proportion of these patients was treated previously with clomiphene-citrate and/or gonadotropins elsewhere. Exclusion criteria were: (1) Thyroid dysfunction (TSH < 0.2 mIU/L or TSH > 4.2 mIU/L), (2) bilateral tubal occlusion on hysterosalpingogram and (3) severe male infertility (sperm concentration $< 10 \times 10^6$). This study was approved by the local Ethics Review Committee of the Dijzigt Academic Hospital and the Erasmus University.

Study design: Initial screening included random transvaginal sonography (TVS) and blood withdrawal. Patients with peri-ovulatory E_2 concentrations (> 400 pmol/L) or luteal P concentrations (P > 15 nmol/L) were excluded. The ratio of T x 100/SHBG was used to calculate the free androgen index (FAI).

Patients were treated by daily i.m. injections with urinary FSH (HMG [Humegon®, N.V. Organon, The Netherlands]) according to a step-down protocol (as described below). Monitoring of ovarian response by TVS was performed every 2 or 3 days, until human chorionic gonadotropin (hCG [Pregnyl®, N.V. Organon, The Netherlands]) was administered. hCG was injected intramuscularly as a single dose of 10 000 IU on the day at least one follicle ≥18 mm was observed upon TVS examination. If more than 3 follicles ≥16 mm were present, stimulation was cancelled and the patient was advised to use barrier contraceptives. Sonographic monitoring was performed using a 6.5 MHz transvaginal transducer (Model EUB-415, Hitachi Medical Corporation, Tokyo, Japan). Monofollicular growth was defined as one follicle ≥16 mm on the day of hCG administration. Treatment was started between cycle day 3 and 5 after a spontaneous or progestagen-induced withdrawal bleeding. The starting dose of gonadotropins was 2 ampules (= 150 IU) per day. The initial dose was increased with ½ ampule per day if ovarian response was absent after 5 days. The first decrease in dose by ½ ampule/day was based on TVS visualization

of a follicle ≥10 mm ('ovarian response'), which coincides with appearance of the dominant follicle during the normal menstrual cycle (Pache *et al.*, 1990). Further dose-decrease, each time by ½ ampule/day, was performed every 3 days to a minimum dose of 1 ampule/day. This dose was continued until the day of hCG administration. If growth of follicles was absent during 10 subsequent days (including one 'step-up' of ½ ampule/day on the 5th day), further medication was withheld and a higher starting dose was used the next cycle. No luteal support was provided.

Statistical analysis: Values given are mean ± standard deviation (SD) unless stated otherwise. *P*-values given are two-sided and 0.05 was considered the limit of statistical significance. Comparisons of categorical data between patient-groups were tested with the χ^2-test. Continuous data were compared using the Mann–Whitney test. Correlation coefficients given were calculated according to Pearson. Ovulation after hCG administration was determined by occurrence of pregnancy, by occurrence of a withdrawal bleeding or by sonography and/or mid-luteal P concentrations (P ≥15 nmol/L) in case of uncertainty based on the former criteria. Pregnancy diagnosis was confirmed by a positive urinary pregnancy test (hCG concentration > 25 IU/L) and ongoing pregnancy rate by sonographic evidence of an intra-uterine gestational sac and fetal heartbeat. Ovarian hyperstimulation was recognized as patients attending our clinic (within 2 weeks following the last administration of gonadotropins) with serious abdominal discomfort and sonographic evidence for grossly enlarged ovaries and increased quantities of free abdominal fluid. Subdivision of the study population using clinical, endocrine and sonographic characteristics was based on calculated values of these parameters in a normal control population as described previously (van Santbrink *et al.*, 1997a).

Assays: Blood samples were obtained through venepuncture and centrifuged within two hours after withdrawal. Serum was stored at −20°C and assayed for FSH and LH by immunoradiometric assay (IRMA) kits provided by Medgenix (Fleurus, Belgium), and androstenedione (AD), sex-hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS) and testosterone (T) by radioimmunoassay (RIA) kits provided by Diagnostic Products Corporation (Los Angeles, CA), as described previously (Fauser *et al.*, 1991). Intra- and inter-assay coefficients of variation were less than 3% and 8% for FSH, less than 5% and 15% for LH, less than 8% and 11% for AD, less than 4% and 5% for SHBG, less than 4% and 6% for DHEAS and less than 3% and 5% for T, respectively.

3.3.3 Results

For patient characteristics after initial screening of the study population see Table 3. Median duration of infertility was 3 years (range, 1 to 14 years). Serum prolactin concentrations were elevated in 4 subjects (4%). Oligomenorrhea was observed in 70 (72%) of all patients and 67 (69%) suffered from a primary infertility. Patients were treated with exogenous gonadotropins according to a step-down protocol with a total number of induction cycles of 286 and a median number of cycles per patient of 3 (range, 1 to 13) cycles. Results of ovulation induction in the total study group are shown in Table 4. Initial clinical, biochemical, and sonographic patient characteristics were compared with treatment results and complication rates (Table 5). Number of ampules needed and duration of gonadotropin administration were influenced by BMI ($r = 0.39$; $P < 0.001$; $r = 0.26$, $P = 0.01$, respectively), initial FAI ($r = 0.24$, $P = 0.03$; P = NS) and initial serum E_2 concentrations ($r = -0.20$, $P = 0.05$; $r = -0.23$, $P = 0.03$). There was a negative correlation between ovulation rate and BMI ($r = -0.20$, $P = 0.05$), DHEAS ($r = -0.23$, $P = 0.03$), FAI ($r = -0.26$, $P = 0.02$), mean ovarian volume ($r = -0.25$, $P = 0.03$) and polycystic ovaries ($r = -0.27$, $P = 0.03$). Serum FSH decreased by age ($r = 0.20$, $P = 0.05$). Cancellation rate was correlated to initial mean ovarian volume ($r = 0.24$, $P = 0.04$), FAI ($r = 0.26$, $P = 0.04$) and DHEAS ($r = 0.24$, $P = 0.02$).

Women who conceived did not differ significantly from women who did not with regard to anamnestic, clinical, endocrine, or sonographic screening parameters (data shown partially in Table 5). Initial screening of mean ovarian volume and DHEAS correlated with mono- or multifollicular development (see Table 3). Initial serum DHEAS also correlated with multiple gestation ($r = -0.32$; $P = 0.04$). In women presenting with multifollicular growth pregnancy rates were higher ($P = 0.03$), but comparison between multiple pregnancy rates did not reach statistical significance.

3.3.4 Discussion

In case initial clinical, endocrine or sonographic screening could predict individual treatment response of anovulatory patients with exogenous gonadotropins this would be an important step forward in the improvement of fertility treatment. It could be the basis for a clinically relevant classification of this patient group: with consequences for choice of treatment strategy, duration of medication, and intensity of monitoring. The present study is a first attempt to determine certain correlations between single initial clinical, endocrine, or sonographic characteristics and treatment response parameters in gonadotropin induction of ovulation.

Table 3 Initial clinical, biochemical and sonographic characteristics (mean ± SD) of a group of normogonadotropic oligo- or amenorrheic clomiphene-citrate resistant infertile women and a sub-division of these patients based upon monofollicular (not more than 1 follicle ≥16 mm) or multifollicular growth on the day of hCG administration during gonadotropin induction of ovulation.

	Total group n = 97	*Monofollicular growth n = 57*	*Multifollicular growth n = 40*
Clinical:			
Age (yrs)	29 ± 4	30 ± 4	28 ± 5*
BMI (kg/m^2)	26 ± 6	26 ± 5	26 ± 6
Amenorrhea (%)	28	23	35
Endocrine:			
FSH (IU/L)	4.3 ± 1.6	4.4 ± 1.6	4.1 ± 1.2
LH (IU/L)	6.7 ± 4.5	7.0 ± 4.6	6.5 ± 4.3
AD (nmol/L)	15.6 ± 6.9	16.0 ± 7.2	15.4 ± 6.9
T (nmol/L)	2.5 ± 1.1	2.5 ± 1.0	2.6 ± 1.2
DHEAS (µmol/L)	7.4 ± 3.9	6.6 ± 3.7	8.6 ± 4.1*
FAI (T x 100/SHBG)	6.3 ± 4.6	5.8 ± 4.0	7.2 ± 5.0
Ultrasound:			
Mean follicle number	13.8 ± 7.3	13.1 ± 7.3	15.6 ± 7.4
Mean ovarian volume (mL)	12.4 ± 5.3	11.3 ± 4.5	14.5 ± 6.1*
Total stroma score	4 ± 1	4 ± 1	4 ± 1

*$P \leq 0.05$ (mono vs. multifollicular growth, significance tested with Mann-Whitney).

Observations in the present study include BMI, initial serum FAI, and E_2 correlating with total number of ampules needed; BMI and serum E_2 correlated with duration of induction; initial serum DHEAS and ovarian volume correlated with multifollicular development; while DHEAS, FAI, polycystic ovaries, and ovarian volume correlated with ovulation rate.

Negative predictive factors of treatment results for ovulation induction as well as for controlled hyperstimulation preceding IVF reported in literature are: (1) advanced patient age (Dor *et al.*, 1980; Padilla *et al.*, 1989; Tan *et al.*, 1992; McClure *et al.*, 1993; Check *et al.*, 1994), (2) obesity (McClure *et al.*, 1992; Chong *et al.*, 1986; Hamilton-Fairley *et al.*, 1992; Kiddy *et al.*, 1992), (3) decreased ovarian reserve (higher basal serum FSH [Muasher *et al.*, 1988; Toner *et al.*, 1991] or low serum E_2 [McClure *et al.*, 1993], poor FSH clomiphene-citrate challenge test [Tanbo *et al.*, 1992] or initial small ovarian volume on ultrasound [Syrop *et al.*,

Table 4 Treatment outcome of gonadotropin ovulation induction in a group of normogonadotropic oligo- or amenorrheic infertile women with clomiphene-citrate resistance or clomiphene-citrate failure and after subdivision in patients presenting with monofollicular (not more than 1 follicle ≥16 mm) or multifollicular growth on the day of hCG administration.

	Total group n = 97	*Monofollicular growth n = 57*	*Multifollicular growth n = 40*
Number of cycles	286	149	137
Max. daily dose (amp.)*	5.5 (1.5–5.5)	5.5 (1.5–5.5)	3.0 (1.5–3)
Total no. of ampules needed*	14 (5–33)	14 (5–33)	14 (7–33)
Treatment duration (days)*	9 (5–20)	9 (5–16)	9 (6–20)
Ovulation rate (%)	83	86	83
Pregnancies (%)	49	44	56†
Mult. pregnancies (%)	17	12	25
Abortions (%)	13	4	25

*Median and range
†$P = 0.03$
None of the remaining comparisons between both groups reaches statistical significance, possibly due to small numbers.

1995; Lass *et al.*, 1997]), and (4) elevated serum androgens (DHEAS, T or FAI [Farhi *et al.*, 1997]).

The effects of advanced patient age described in literature are: less successful stimulation, reduced pregnancy rates (Dor *et al.*, 1980), and reduced live birth rates (Padilla *et al.*, 1989; Tan *et al.*, 1992; McClure *et al.*, 1993; Check *et al.*, 1994). We were not able to detect any of these consequences of patient age for results of step-down induction of ovulation in the present study group (Table 5), although a tendency was observed that older age was associated with more patients exhibiting monofollicular growth (Table 3).

Obesity has also been correlated with negative treatment outcome. In the present study, as in literature, obese patients needed more gonadotropins and a prolonged induction period (McClure *et al.*, 1992; Chong *et al.*, 1986), but reduced ovulation rates and ongoing pregnancy rates (Hamilton-Fairley *et al.*, 1992) could only be confirmed for the former issue (Table 5). Negative influence of obesity on treatment results of ovulation induction has been described as being reversible with weight reduction (Kiddy *et al.*, 1992).

Reduced ovarian reserve (high basal cycle day 3 FSH [Muasher *et al.*, 1988; Toner *et al.*, 1991], or excessive FSH response to clomiphene-cit-

Table 5 Treatment outcome and division in several arbitrarily defined subgroups of 97 normogonadotropic oligo- or amenorrheic clomiphene-resistant infertile women treated with exogenous FSH for ovulation induction.

		%	*Amp. FSH*	*Dur. FSH*	*Monofoll. growth %*	*Ovulation %*	*Pregn. %*	*Multiple Pregn.%*	*Abort %*
Age	<35	87	16±8	9±3	60	82	45	17	12
	≥35	13	17±6	11±3	69	91	46	17	17
BMI	<26	56	13±5	9±2	64	85	44	17	13
	≥26	44	19±9***	10±3**	59	81*	54	17	13
LH	normal	63	16±8	10±3	57	82	44	19	7
	high	37	15±8	9±2	69	85	47	15	20
Androgens†	normal	57	15±6	9±3	63	81	42	14	14
	high	43	17±8	10±3	54	83	56	23	14
FAI	<9.3	76	15±7	9±3	61	88	45	13	10
	≥9.3	24	18±7*	10±2	47	63*	50	17	20
Polycystic ovaries‡	no	25	14±6	9±2	75	98	47	13	13
	yes	75	16±7	10±3	61	77*	45	17	17

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ (significance of association calculated with χ^2 test or Mann-Whitney).
†Increased AD and/or T serum concentrations, as previously described (see ref # 5).
‡Increased ovarian volume and/or follicle number, as previously described (see ref # 5).

rate challenge test [Tanbo *et al.*, 1992]) has been described as being a problem especially for controlled hyperstimulation in IVF resulting in less ovarian responsiveness and reduced pregnancy rates. For gonadotropin induction of ovulation the impending ovarian failure problem has not been described related to FSH concentrations but to basal E_2 concentrations (McClure *et al.*, 1993). In the present study, low serum E_2 concentrations are observed to be related to an increased amount of gonadotropins needed and a longer induction period. In the literature an association with increased miscarriage rates was also described (McClure *et al.*, 1993). A different approach may be to determine ovarian reserve by ovarian volume on ultrasonography. Indeed, decreased ovarian volume is associated with poor IVF outcome: less oocytes and embryos (Syrop *et al.*, 1995) or increased total amount of gonadotropins needed, higher cancellation rate due to poor response, and less follicles and oocytes obtained (Lass *et al.*, 1997). Smaller initial ovarian volume in the present ovulation induction study was associated with lower initial serum E_2, less preovulatory follicles (more monofollicular growth) but higher cancellation rates which confirms findings in IVF studies. Patient age did not match with above-mentioned parameters for ovarian aging but was associated with a higher percentage of monofollicular growth.

Good responders in ovulation induction (patients treated according to a low-dose step-up regimen with high FSH:LH ratio and increased numbers of follicles ≥8 mm on treatment day 8) were prone to develop multifollicular growth (Farhi *et al.*, 1997). This may be partly explained by accumulation of FSH in the late follicular phase (van Santbrink *et al.*, 1997b). Multifollicular growth and high preovulatory E_2 concentrations in ovulation induction were reported not to be associated with multiple gestation (Goldenberg *et al.*, 1994) but did correlate with changes in ovarian hyperstimulation (Blankstein *et al.*, 1987). These findings are in concordance with observations in the present study: the number of preovulatory follicles on the day of hCG administration was correlated with higher pregnancy rates but did not correlate with higher multiple gestation rates. Since no severe ovarian hyperstimulation did occur in the present study, no conclusions can be drawn on this subject.

Patients with elevated androgens (DHEAS, T or FAI) in a recurrent miscarriage group presented with higher chances of miscarriage in a subsequent spontaneous pregnancy (Tulppala *et al.*, 1993). Surprisingly, single sample LH serum concentrations in the above mentioned study had no predictive value for pregnancy outcome in this particular group. This is in contrast with earlier reports in which high LH was suggested as a risk factor for miscarriage (Regan *et al.*, 1990; Hamilton-Fairley *et al.*, 1991; Balen *et al.*, 1993). Risk factors for miscarriage could not be identified in

our study group (Table 5). This may be due to the relatively small number of patients included. Elevated androgens (FAI and DHEAS) in the present ovulation induction study were associated with decreased ovarian response (more gonadotropins needed, decreased ovulation rate, and higher cancellation rate).

In conclusion this study suggests that obesity, ovarian aging (low basal E_2 serum concentrations and small initial ovarian volume), polycystic ovaries, and elevated serum androgens (DHEAS and FAI) may be associated with impaired treatment response in a group of normogonadotropic oligo- or amenorrheic infertile women with clomiphene-citrate resistance or failure, treated with exogenous gonadotropins according to a step-down dose regimen: more gonadotropins are needed for a prolonged period and ovulation rates are lower.

4

Gonadotropin induction of ovulation

4.1 GENERAL INTRODUCTION

Induction of ovulation using exogenous gonadotropins is generally indicated in patients with normogonadotropic anovulatory infertility who failed to ovulate or conceive during previous antiestrogen treatment. Alternatively, ovarian cautery by laser may also be applied. The aim of this treatment modality is to approach normal conditions as closely as possible, i.e. maturation and ovulation of a single dominant follicle and subsequent singleton pregnancy. However, although gonadotropin therapy has been shown to be rather successful in terms of ovulation rates (reported in literature between 60–100%) and cumulative pregnancies (reported between 20–75%), complication rates are high. Major complications include multiple pregnancies and ovarian hyperstimulation. As these complications have been shown to be related to the magnitude of multi-follicle development, efforts have been made to reduce the number of follicles that reach full maturity. Our group has focused on a 'step-down' dose regimen that more closely mimics FSH serum concentrations during the follicular phase of the normal menstrual cycle. Previous work (Schoot *et al.*, Thesis 1995) has been the basis for development and modification of the step-down dose regimen. It was proposed on the basis of observations in a limited patient group that a step-down dose regimen for induction of ovulation using exogenous gonadotropins could be an effective treatment alternative and may lead to reduced complication rates (Schoot *et al.*, Thesis 1995). The objective of the following studies was to investigate the clinical usefulness of a gonadotropin step-down dose regimen for induction of ovulation in normogonadotropic clomiphene-resistant anovulation on a large scale and to compare this treatment protocol in randomized prospective fashion with a low-dose step-up protocol. The objective in the latter study was to assess potential differences in treatment duration and treatment dose, ovarian stimulation (late follicular FSH serum profile) and response (serum E_2 concentrations and number of dominant follicles on the day of hCG administration).

4.2 *GONADOTROPIN INDUCTION OF OVULATION USING A STEP-DOWN DOSE REGIMEN: SINGLE-CENTER CLINICAL EXPERIENCE IN 82 PATIENTS*

4.2.1 Introduction

Since the introduction of urinary gonadotropins in clinical medicine thirty years ago, this treatment modality has been the primary choice for infertile patients suffering from clomiphene-resistant normogonadotropic anovulation. Although indisputably effective, these compounds carry important risks, chiefly ovarian hyperstimulation (Stephenson, 1991; Navot *et al.*, 1992) and multiple pregnancies (Derom *et al.*, 1993), associated with considerable obstetric complications (Levene *et al.*, 1992; Callahan *et al.*, 1994).

In the past, various treatment schedules have been reported including single dose, intermittent or multiple fixed doses, as well as incremental or decremental dose regimens (Taymor *et al.*, 1967; Thompson *et al.*, 1970). At present, the most frequently used administration schedules are 'step-up' regimens, i.e. increasing doses of exogenous gonadotropins through daily i.m. injections until ovarian response is considered to be 'sufficient'. In an attempt to diminish complication rates, conventional step-up regimens were modified to 'low-dose, step-up' regimens (Seibel *et al.*, 1984; Buvat *et al.*, 1989; Hamilton-Fairley *et al.*, 1991) starting with a lower initial dose (½ or 1 ampule/day) and with small increments (½ ampule) at weekly intervals. Using low-dose regimens, improved clinical outcome was reported compared to conventional step-up regimens.

The half-life of exogenous urinary FSH in females has been reported to be ~44 h (Diczfalusy *et al.*, 1989; Mannaerts *et al.*, 1993), and if equal daily doses are administered, steady state serum FSH concentrations are reached after 5 to 7 days (Mizunuma *et al.*, 1990; Schoot *et al.*, 1994; van Heusden *et al.*, unpublished data). It should therefore be considered that during step-up regimens elevated FSH serum concentrations may occur during the late follicular phase which may interfere with selection of a single dominant follicle (Fauser *et al.*, 1993b; Fauser, 1994; van der Meer *et al.*, 1994). It has been shown that the magnitude of FSH accumulation determines ovarian (hyper)response (Ben Rafael *et al.*, 1986). In contrast, in the normal menstrual cycle the dominant follicle shows a decreased dependency of FSH (Messinis *et al.*, 1990; Hall *et al.*, 1991; van Santbrink *et al.*, 1995), and reduced FSH concentrations during the late follicular phase were found to be essential for monofollicular development in the monkey model (Zeleznik *et al.*, 1985). Our previous studies have shown that step-down gonadotropin dose regimens result in a significant (40%) reduction in serum FSH concentrations (Schoot *et al.*,

1995) and closely resemble decreasing FSH concentrations in spontaneous cycles (van Santbrink *et al.*, 1995). This prospective, single-center, non-comparative study was undertaken to investigate the clinical usefulness of a gonadotropin step-down regimen for the induction of ovulation in women suffering from clomiphene-resistant anovulation.

4.2.2 Patients and methods

Between 1991 and 1993, 82 patients attending our Fertility Clinic presenting with oligomenorrhea (cycle length >35 days) or amenorrhea (no vaginal bleeding for at least 6 months), FSH serum concentrations within normal limits (between 1 and 10 IU/L) and infertility (duration median 3 years, range, 1 to 12 years) were included in this study. Included patients had a median age of 29 years (range, 20 to 42 years), body mass index (BMI, weight divided by height squared) was 24 kg/m^2 (range, 19 to 42 kg/m^2), median luteinizing hormone (LH) concentration was 5.2 IU/L (range, 1.1 to 14.5 IU/L) and testosterone (T) concentration was 1.9 nmol/L (range, 0.4 to 5.8 nmol/L). The protocol was approved by the Human Subject Committee of the Dijkzigt Academic Hospital / Erasmus University. All participants suffered from clomiphene-resistant anovulation, which was defined as failure to ovulate or to conceive, despite increasing doses of clomiphene citrate (up to 150 mg/day, from cycle day three to seven) during at least 3 consecutive cycles. Since this clinic is a tertiary referral center, a proportion of these patients was previously treated with gonadotropins elsewhere. Exclusion criteria were; (1) hypergonadotropic (FSH >10 IU/L) or hypogonadotropic (FSH <1 IU/L) anovulation, (2) anovulation in the presence of thyroid dysfunction (thyroid-stimulating hormone <0.2 or >4.2 mU/L) or hyperprolactinemia (prolactin >15 nmol/L), (3) bilateral tubal occlusion on hysterosalpingogram, and (4) severe male infertility (sperm count < 10 x 10^6 /mL). The following additional fertility problems were included in this study; cervical hostility in 8 cycles (3.3%) and male subfertility (sperm count 10–40 x 10^6 spermatozoa/mL, motility <50%, or both) in 18 cycles (7.6%). Infertility was primary in 56 patients (68%), while a secondary infertility was found in 26 patients (32%).

Patients were treated by daily intramuscular injections with gonadotropins (HMG; Humegon®, NV Organon Int., Oss, The Netherlands) in 93% of cycles and at random with purified urinary FSH (Metrodin®, Serono, Weesp, The Netherlands) in 7% of cycles according to a step-down dose regimen (as described below). Monitoring of ovarian response by transvaginal sonography (TVS) was performed every 2 or three days, until human chorionic gonadotropin (hCG; Pregnyl®, NV Organon Int.) was administered. Sonographic monitoring was performed using a 6.5 MHz

transvaginal transducer (Model EUB-415; Hitachi Medical Corporation, Tokyo, Japan). At the beginning of this study, in 43 cycles (18% of total cycles) patients were pretreated with gonadotropin-releasing hormone (GnRH) agonists (Buserelin; Suprefact®, Hoechst, Amsterdam, The Netherlands]) for 3 weeks (3 x 400 µg/day intranasally), which was continued during gonadotropin administration until the day of hCG administration (Schoot *et al.*, 1992b).

For each patient, blood samples for initial screening were randomly taken through vein puncture, centrifuged within 2 h of withdrawal and stored at −20°C until assayed. Serum was assayed for immunoreactive FSH, LH, T and Progesterone (P), as described previously (Fauser *et al.*, 1991).

Step-down protocol: Treatment was started between cycle days 3 and 5 after a spontaneous or progestagen-induced withdrawal bleeding. The starting dose of gonadotropins was dependent on the BMI and patients were divided into 3 groups: (i) BMI 19–23 kg/m^2 (n = 41; 50%), 1½ ampule (= 113 IU) per day; (ii) BMI 23–28 kg/m^2 (n = 28; 34%), 2 ampules (= 150 IU) per day; and (iii) BMI >28 kg/m^2 (n = 13; 16%), 2½ ampules (= 188 IU) per day. This approach was based on the observation that following a single dose of gonadotropins, maximum serum FSH concentrations were dependent on body weight (Mannaerts *et al.*, 1993). The first decrease in dose by ½ ampule/day, was based on sonographic visualization of a follicle >9 mm, which coincides with appearance of the dominant follicle during the normal menstrual cycle (Pache *et al.*, 1990). The initial dose was increased with ½ ampule/day if ovarian response was absent after 5 days. Further dose-decrease, each time by ½ ampule/day, was performed every 3 days to a minimum dose of 1 ampule/day. This dose was continued until the day hCG could be administered. During the follicular phase, the continued growth of follicles was monitored by TVS. hCG was injected i.m. as a single dose of 10 000 IU on the day that at least one follicle ≥18 mm was present upon TVS examination. If there were more than 3 follicles ≥16 mm in diameter, stimulation was cancelled and the patient was advised to use barrier contraceptives. If the growth of follicles was absent during 10 subsequent days (including one 'step-up' of ½ ampule/day on the 5th day), further medication was withheld and a higher starting dose was used the next cycle. No luteal support was provided.

Data analysis: Values given are mean ± SD unless stated otherwise. Because no significant difference was found in clinical and sonographic parameters between the group with and without GnRH agonist co-treatment and patients treated with HMG or pFSH (tested using the

Mann–Whitney or Wilcoxon test), results were pooled. *P* values given are two-sided, and 0.05 was considered the limit of statistical significance. Differences between patient groups were tested using the Mann–Whitney or Student's *t*-test. The follicular phase was arbitrarily defined as the interval between first day of gonadotropin medication and the day following hCG administration (hCG + 1; presumed ovulation). The luteal phase was defined as the interval between day of hCG + 1 and the first day of subsequent menses. Distribution of ovarian follicles was arbitrarily classified in categories according to diameter: 10–12, 12–16 and ≥16 mm. Ovulation after hCG administration was determined by a viable pregnancy, the occurrence of withdrawal bleeding or sonography and/or mid-luteal P concentrations in case of uncertainty of the former criteria. Pregnancy diagnosis was confirmed by a positive urinary pregnancy test (hCG concentration >25 IU/L) and ongoing pregnancy rate by sonographic evidence of an intrauterine gestational sac and a fetal heart action. Ovarian hyperstimulation was recognized in patients attending our clinic (within 2 weeks following the last administration of gonadotropins) with serious abdominal discomfort and sonographic evidence for grossly enlarged ovaries and increased quantities of free abdominal fluid.

4.2.3 Results

In all, 82 patients were treated with exogenous gonadotropins for a total of 234 cycles. The median cycle number per patient was 2 (range, 1 to 7). Co-treatment with GnRH analogues was used in 43 initial cycles (18% of total); no statistically significant differences between the group with and without co-treatment were found with regard to the duration of stimulation (11.3 vs. 10.5 days), the total number of ampules of gonadotropin used per cycle (15.5 vs. 13.0 ampules), the luteal phase length (12.4 vs. 13.3 days), and the pregnancy rate (5/43 vs. 32/191). In 16 cycles (7%), purified FSH was given instead of HMG; no statistically significant differences in both treatment groups with regard to treatment outcome were found (data not shown). In 36 (15%) cycles the initial dose of HMG was increased.

In the overall study group the duration of ovarian stimulation until ovulation was 10.6 ± 3.6 days and 13.5 ± 6.9 ampules were administered. The luteal phase had a duration of 13.1 ± 4.2 days. For more detailed information see Figure 10. hCG was given in 214 (92%) cycles. One cycle was considered to be anovulatory because of low mid-luteal P concentrations (2 nmol/L). In 10 (4%) cycles hCG was withheld because of cancellation of gonadotropin medication due to a lack of ovarian response. In the additional 10 (4%) cycles more than 3 follicles ≥16 mm

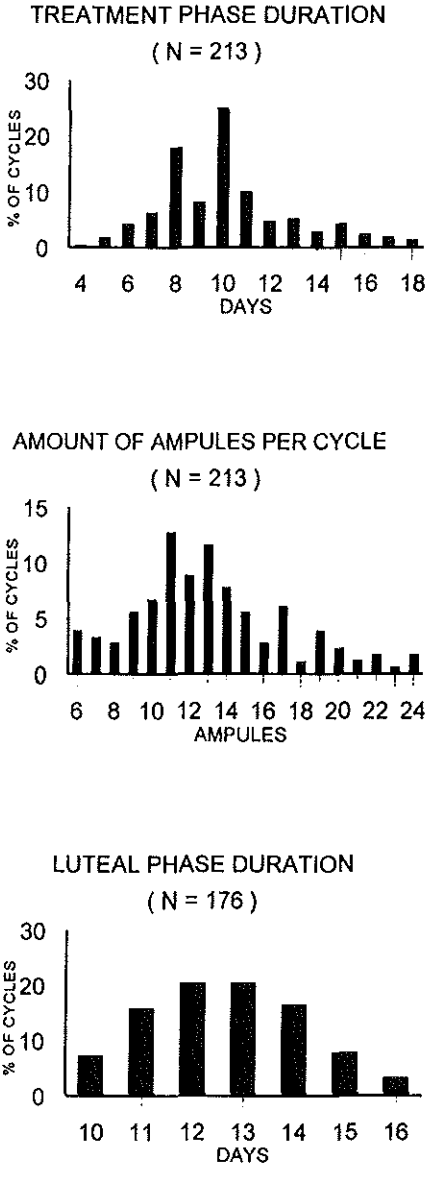

Figure 10 Distribution of duration of treatment phase (duration of initiation of gonadotropin medication until one day following hCG administration *(top)*; the required number of ampules of gonadotropins per cycle *(middle)*; and the duration of the luteal phase *(bottom)* in a total of 213 ovulatory cycles using a step-down dose regimen for induction of ovulation in 82 clomiphene-resistant anovulatory women (outlyers are excluded).

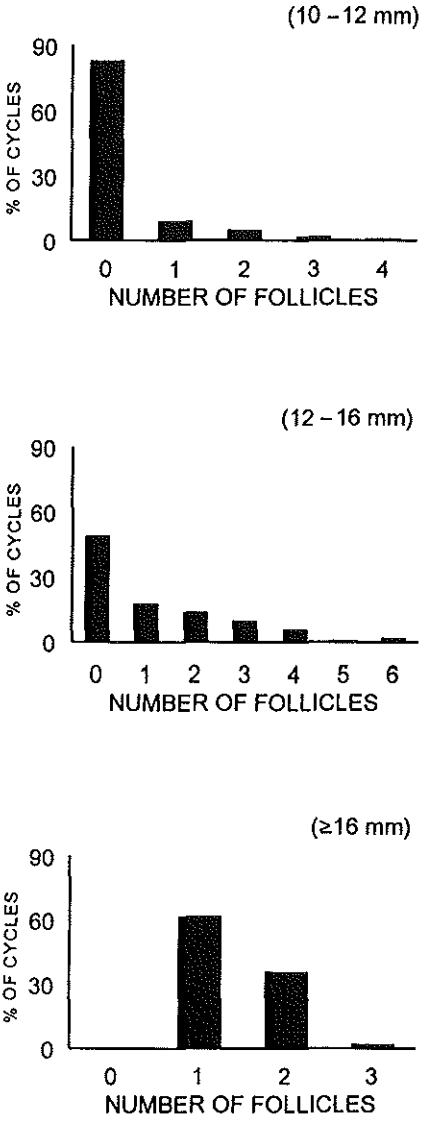

Figure 11 Distribution of number of follicles of 10–12 mm *(top)*; 12-16 mm *(middle)*; and ≥16 mm *(bottom)* in diameter on the day of hCG administration in 213 ovulatory cycles following a gonadotropin step-down dose regimen for induction of ovulation in 82 clomiphene-resistant anovulatory women.

were present. The ovulation rate was 91%. On the day of hCG administration the diameter of the leading follicle was 19.5 ± 1.6 mm, there were 1.4 ± 0.7 follicles ≥16 mm in diameter per ovulatory cycle, in 131 (62%) cycles there was only one follicle ≥16 mm (monofollicular growth), and in 208 (98%) cycles one or two follicles ≥16 mm were present (Figure 11). In 39 cycles (17%), luteal serum P concentrations were determined: the median value was 50 nmol/L. There were 4 cases of ovarian hyperstimulation (1.7%), for which in 2 cases clinical attendance (conservative management) was necessary for severe abdominal discomfort. In one of these patients, ovarian hyperstimulation developed although hCG was withheld. In this patient the numbers of larger (≥16 mm) and intermediate (12–16 mm) follicles were 8 and 4, respectively. In the other three patients the mean numbers of larger (≥16 mm) and intermediate (12–16 mm) follicles were 2 and 8 on the day of hCG administration. None of these four women were pregnant. The overall pregnancy rate per ovulatory cycle was 47% after 7 months, as determined by life-table analysis. The pregnancy rate per cycle was 16% for all started cycles and 17% for ovulatory cycles. A total of 37 pregnancies occurred, of which 2 were twins and 1 was triplet (multiple pregnancy rate of 8%). A viable pregnancy was confirmed by sonography in 30 (81%) of the 37 chemical pregnancies. The ongoing pregnancy rate was 13% per started induction cycle and 14% per ovulatory cycle. The overall singleton ongoing pregnancy rate was 12% per cycle and 13% per ovulatory cycle (see also Table 6).

No significant differences were found between non-pregnant and pregnant women with regard to age, BMI, LH concentration, T concentration or the number of follicles present in the ovary before treatment (data not shown).

4.2.4 Discussion

Although effective, the induction of ovulation using exogenous gonadotropins in step-up dose regimens is still associated with considerable complications, chiefly multiple pregnancies and ovarian hyperstimulation. In this respect, low-dose step-up regimens have been advocated recently, which offer the advantage of reduced complication rates. However, the latter dose regimen seems to be at the cost of a prolonged duration of medication and an overall decrease in pregnancy rates, at least in the hands of some clinical investigators (Hull, 1991). It should also be mentioned that even during low-dose step-up protocols, a significant incidence of ovarian hyperstimulation has been reported (Buvat *et al.*, 1989; Herman *et al.*, 1993). The low-dose step-up approach is based on the FSH

Table 6 Comparison of ovarian response and clinical outcome following three different dose regimens for gonadotropin induction of ovulation.

	Step-up		*Low-dose, step-up*				*Step-down*
	Dor (1980)	*Hull[a] (1991)*	*Buvat (1989)*	*Hamilton-F. (1991)*	*Hull[b] (1991)*	*Balen (1994)*	*vSantbrink (1995)*
Study design	single-center comp.[c]	review non-comp.[d]	single-center comp.[c]	single-center non-comp.[d]	review non-comp.[d]	single-center non-comp.[d]	single-center non-comp.[d]
Number of patients	348	111	23	100	144	103	82
Number of cycles	1144	210	44	401	459	603	234
Duration treatment phase (days)	n.r.[e]	n.r.	16	14	n.r.	n.r.	11
Ampules per cycle	n.r.	n.r.	19	19	n.r.	n.r.	14
Ovulation rate (%)	n.r.	78	70[f]	72[g]	74	68[h]	91[i]
'Monofollicular' cycles							
% of ovulatory cycles:	n.r.	n.r.	84[j]	73[k]	n.r.	n.r.	62[l]
% of all started cycles:	n.r.	n.r.	59[j]	55[k]	n.r.	n.r.	56[l]
Pregnancy rate (%):							
*per started cycle	7	23	16	11	11	14	16
*per ovulatory cycle	n.r.	30	23	16	15	20	17
Cumulative pregn. rate (%)	50	n.r.	n.r.	55	n.r.	73[m]	47
Multiple pregn. rate (%)	n.r.	23	0	4	11	18	8
Ongoing singleton pregn. rate (%)	n.r.	17	14	7	10	9	12
Abortion rate (%)	n.r.	17	14	32	39	16	19
OHSS rate (%)	n.r.	n.r.	5	1	n.r.	1	2

[a]Combined data of 6 single-center studies; [b]combined data of 6 single-center studies; [c]study was not prospective, randomized; [d]non-comp. = non-comparative; [e]n.r. = not reported; [f]21% cancelled due to multifoll. development; [g]cancellation only due to multifoll. development (low progesterone and no ovarian response 24%); [h]cancelled due to multifoll. development (8%), non-response (8%) and no ovulation (16%); [i]cancelled due to multifoll. development (4%) + non-response (4%) and low progesterone (=1%); [j]1 follicle ≥15 mm on day of hCG administration; [k]1 follicle ≥18 mm on day of hCG administration; [l]1 follicle ≥16 mm on day of hCG administration; [m]after 12 cycles.

'threshold' concept, introduced in 1978 by Brown (Brown, 1978), indicating that a threshold concentration of serum FSH should be surpassed to elicit an ovarian response. Multifollicular growth might be associated with a higher (and prolonged) elevation of FSH concentrations above the threshold (van der Meer *et al.*, 1994). The FSH 'gate' (Baird *et al.*, 1987) or FSH 'window' (Fauser *et al.*, 1993a; Fauser, 1994) concept adds the element of time to the threshold theory. The longer the threshold is surpassed, the more follicles will be stimulated to ongoing growth and dominant follicle development.

The 'step-down' approach for gonadotropin induction of ovulation was subsequently developed aiming at serum FSH concentrations to be above the threshold for just a restricted period of time, resulting in a narrow FSH window. However, the FSH threshold may vary considerably in PCOS patients, which makes it difficult to choose an adequate starting dose. It was hypothesized that the dominant follicle will continue its development due to an enhanced sensitivity for FSH, whereas serum FSH concentrations fall below the threshold for less mature follicles. In a previous study by our group, serum FSH concentrations were monitored daily in a subgroup of 22 patients using a similar step-down gonadotropin dose regimen (with GnRH agonist co-treatment), which did confirm the occurrence of a significant ($P = 0.001$) decrease (10% per day for 4 days) in serum FSH concentrations from median maximum concentrations of 7.6 IU/L to 4.7 IU/L on the day of hCG administration (Schoot *et al.*, 1995). Other groups have reported that cycles prone to develop ovarian hyperstimulation could be 'rescued' by withholding HMG for several days (Rabinovici *et al.*, 1987). Hence, a late and pronounced decrease in serum FSH concentrations prevents the further development of medium-sized follicles, whereas large follicles still ovulate following delayed hCG administration. This approach, presently referred to as 'coasting', in fact closely resembles a step-down regimen. Mizunuma *et al.* (1991) reported a small and uncontrolled study ($n = 9$) using a step-down regimen. A short mean stimulation phase (7.4 days), a pregnancy rate per cycle of 16% (multiple pregnancy rate of 13%), and a hyperstimulation rate of 7% were reported. The small number of patients and the selection criteria used to include patients (admission for step-down treatment after successful induction of ovulation during a step-up regimen in the preceding cycle) limit the conclusions that can be drawn from this study. Also, no explanation was provided for the high cancellation rate of 20%. Preliminary data were reported recently (Steinkampf *et al.*, 1993) from a large ($n = 175$) randomized prospective multicenter study comparing a step-down regimen with an ascending dose schedule. The mean numbers of follicles ≥16 mm in diameter on the day of hCG administration (at least 1 follicle ≥16 mm in

diameter) were similar (1.5 follicle) in both groups tested. Ovulation and complication rates were equal, but pregnancy rates in the step-down group were significantly reduced (7.1 compared to 15.7% in the ascending dose group). However, the used fixed starting dose of 3 ampules/day may have been too high (Schoot *et al.*, 1992b), and a fixed decrease of the dose to 1 ampule/day after 3 days does not take into account individual differences in the FSH threshold (Brown, 1978; Polson *et al.*, 1987; Fauser *et al.*, 1993a) and may result in serum FSH concentrations which drop too early and too rapidly. These considerations may explain the reported poor treatment outcome of the step-down dose regimen. For a comparison of clinical results of the presently applied decremental dose regimen versus (low dose) step-up regimens reported in the literature, see Table 6.

In conclusion, our study shows for the first time that a step-down regimen for gonadotropin induction of ovulation can serve as a safe and successful treatment alternative in patients with clomiphene-resistant anovulation. We feel that the presented data using the step-down approach, with starting doses of 1½ to 2½ ampules/day based on body weight, demonstrate convincingly that follicles grow and ovulate and pregnancies occur, and therefore challenge the dogma of step-up regimens. The duration of stimulation, the amount of gonadotropins needed, and the clinical outcome (in terms of both success and complication rates) appear favorable. It should be stressed, however, that observations from non-comparative studies should be interpreted with great care. Statistically meaningful comparisons between different studies cannot be made due to: (i) the non-comparative nature of the great majority of studies where larger patient numbers are involved; (ii) selection bias due to different patient populations studied; in this study, for example, exclusion criteria were less strict than in most reported studies (Hamilton-Fairley *et al.*, 1991), and because our clinic is a tertiary referral center some patients were treated unsuccessfully elsewhere before attending our clinic; (iii) differences in monitoring of ovarian response; and (iv) different criteria being used for calculating success and complication rates. Therefore, one should be cautious with the clinical introduction of this dose regimen. At present, the step-down approach may be reserved for PCOS women who exhibit pronounced multiple follicle development during step-up regimens. Because of ongoing concern with regard to the increased incidence of multiple gestation and the resulting complications (Callahan *et al.*, 1994), which are largely due to the induction of ovulation (Derom *et al.*, 1993), we strongly believe that this approach deserves further consideration.

4.3 *URINARY FOLLICLE-STIMULATING HORMONE FOR NORMOGONADOTROPIC CLOMIPHENE-RESISTANT ANOVULATORY INFERTILITY: PROSPECTIVE, RANDOMIZED COMPARISON BETWEEN LOW-DOSE STEP-UP AND STEP-DOWN DOSE REGIMENS*

4.3.1 Introduction

Since the early 1960s, infertile patients with anovulation have been treated with human menopausal gonadotropin and human chorionic gonadotropin (hCG). This treatment modality has proven to be effective (Ginsburg *et al.*, 1991), although associated complications (principally multiple pregnancies and ovarian hyperstimulation) remain an issue of major concern (Sagle *et al.*, 1991; Navot *et al.*, 1992; Levene *et al.*, 1992; Fauser *et al.*, 1997a). Based on Brown's theory (Brown, 1978), conventional step-up regimens have been modified to a low-dose step-up protocol (Buvat *et al.*, 1989; White *et al.*, 1996). A lower initial dose of a ½ or 1 ampule/day is used, and the dose is increased by small increments (½ ampule) at 1 to 2-week intervals in an attempt to slowly and prudently surpass the individual FSH threshold. In both treatment regimens the administered dose of gonadotropins is kept constant from the day of sufficient ovarian response on ultrasound (Buvat *et al.*, 1989; Sagle *et al.*, 1991; White *et al.*, 1996), until the day of hCG administration to induce ovulation. The risk of multiple follicle development and subsequent complications is reduced with low-dose step-up as compared to conventional step-up regimens (Buvat *et al.*, 1989; White *et al.*, 1996). However, low-dose step-up regimens may also lead to multiple follicle development (Herman *et al.*, 1993) and appear to be more time consuming (Fauser *et al.*, 1997a). Due to the long half-life of FSH (Mizunuma *et al.*, 1991; Mannaerts *et al.*, 1993), fixed daily doses of gonadotropin preparations may result in accumulation and, therefore, increased FSH serum concentrations in the late follicular phase. It has been reported that the magnitude of FSH accumulation determines ovarian hyperresponse in patients treated with *in vitro* fertilization (Ben-Rafael *et al.*, 1986).

In contrast, during the normal menstrual cycle the dominant follicle continues to grow despite decreasing FSH serum concentrations (Messinis *et al.*, 1990; Hall *et al.*, 1991; van Santbrink *et al.*, 1995a). Diminishing concentrations of FSH during the follicular phase have been shown to be essential for monofollicle development in the monkey-model (Zeleznik *et al.*, 1985) as well as in the human (Lolis *et al.*, 1995). It is possible that elevated late follicular phase FSH concentrations during step-up regimens unintentionally interfere with single dominant follicle selection (Fauser, 1994). Our previous studies have shown that a step-down gonadotropin

dose regimen more closely resembles decreasing FSH concentrations found in spontaneous cycles (van Santbrink *et al.*, 1995a; Schoot *et al.*, 1995; van Dessel *et al.*, 1995), and can serve as a safe and successful treatment alternative for women suffering from clomiphene-resistant anovulation (van Santbrink *et al.*, 1995b). This study is the first prospective randomized comparison between a low-dose step-up and a step-down protocol for gonadotropin induction of ovulation. The objective was to assess potential differences in treatment duration and dose, ovarian stimulation, and response.

4.3.2 Patients and methods

Subjects: Inclusion criteria were: infertility (seeking pregnancy ≥1 year), cycle abnormalities (oligomemorrhea [interval between menstrual bleedings >35 days] or amenorrhea [no bleeding for at least 6 months]), FSH serum concentration within the normal range (FSH 1–10 IU/L), clomiphene-resistance (defined as failure to ovulate or conceive after clomiphene treatment up to a daily dose of 150 mg from cycle day 3 to 7 during at least 3 consecutive cycles), age 20–40 years, normal PRL (<15 nmol/L) and TSH (0.2 mIU/L < TSH < 4.2 mIU/L) serum concentrations, absence of evidence of bilateral tubal occlusion as assessed by hysterosalpingogram, and absence of severe oligospermia (sperm count $<10 \times 10^6$ spermatozoa/mL).

Thirty-seven patients attending our Fertility Clinic fulfilling above mentioned criteria were included in this study. Patients included (see Table 7) had a median age of 29 years (range, 20 to 40 years), median body mass index (weight divided by height squared) was 25 kg/m^2 (range, 18 to 41 kg/m^2), and the median duration of infertility was 3 years (range, 2 to 7 years). The protocol was approved by the local human subject committee and informed consent was obtained from all participants.

Study design: This was a prospective, randomized, single-center study comparing a low-dose step-up with a step-down dose regimen for induction of ovulation with urinary FSH. Initial screening was performed within 2 months before inclusion. Initial screening included random blood withdrawal and transvaginal sonography (TVS). Sonographic monitoring was performed by a single observer (EJPvS), using an ultrasound machine (Model EUB-415, Hitachi Medical Corporation, Tokyo, Japan) with 6.5 MHz transvaginal transducer. The ovaries were localized in relation to the iliac vessels. Follicles appeared as round or ovoid translucent structures ≥2 mm in diameter. Follicle number was established by scanning each ovary from the inner to the outer margin in longitudinal cross-section. The ovarian volume was estimated according to the following formula:

½(AxBxC), where A is the longitudinal diameter, B the antero-posterior diameter and C the transverse diameter of the ovary. Mean follicle number and mean ovarian volume were calculated from the sum of the left and right values divided by two. Ovarian stroma echogenicity was scored as 1 (normal), 2 (moderately increased), and 3 (markedly increased) as described by Pache *et al.* (1990). Total stroma count was the combined stroma score of both ovaries. For each patient, blood samples for initial screening were randomly taken through venepuncture, centrifuged within 2 h after withdrawal, and stored at −20°C until assayed. Before entering the protocol, serum from each patient was assayed for immunoreactive FSH, LH (normal range 1.3–6.9 IU/L [van Santbrink *et al.*, 1997a]), E_2, testosterone (T), androstenedione (AD), sex-hormone binding globulin (SHBG) and progesterone (P) as described previously (Fauser *et al.*, 1991). After inclusion, patients were randomized by receiving a study number corresponding to a sealed envelope containing the protocol to be followed.

Gonadotropin treatment was started 3–5 days after the initiation of a spontaneous or progestagen-induced withdrawal bleeding. Patients received daily i.m. injections of purified urinary FSH (Follegon®, NV Organon, Oss, The Netherlands) from a single batch (no.: 74029003). Monitoring of ovarian response by TVS was performed every 2 or 3 days until hCG (Pregnyl®, NV Organon) was administered. hCG was injected i.m. as a single dose of 10 000 IU on the day upon which at least one follicle ≥18 mm in diameter was observed by TVS examination. If more than 3 follicles ≥16 mm were present, stimulation was cancelled, and the patient was advised to use barrier contraceptives. During the study, blood samples were obtained on the day treatment was initiated, the day of sufficient ovarian response, the day of hCG injection, and 3 and 7 days thereafter. Sufficient ovarian response was defined as visualization of a follicle ≥10 mm by TVS, which coincides with the appearance of the dominant follicle in the normal menstrual cycle (Pache *et al.*, 1990; van Santbrink *et al.*, 1995a). FSH injections were administered between 0800–1200 h. Blood withdrawal was performed just before gonadotropin injection, i.e. 24 h after the previous injection. During treatment, the ovarian response was monitored by TVS only.

Low-dose step-up protocol: The starting dose of FSH was 1 ampule (= 75 IU) per day. The first increase in dose by a ½ ampule/day was based on absence of a follicle ≥10 mm in diameter after 14 days. Thereafter, the dose was increased by a ½ ampule/day every 7 days if an ovarian response was lacking. If a sufficient ovarian response was observed, the dose was kept constant until the administration of hCG. No luteal support was provided.

Step-down protocol: The starting dose of FSH was 2 ampules (= 150 IU) per day. The first decrease in dose by a ½ ampule/day was based on visualization of at least 1 follicle ≥10 mm in diameter. The initial dose was increased by a ½ ampule/day if an ovarian response remained absent after 5 days. If follicular growth remained absent over the following 10 days (2 incremental steps of a ½ ampule/day), further medication was withheld and the cycle was cancelled. A further dose decrease, each time by a ½ ampule/day, was performed every 3 days to a minimum dose of 1 ampule/day if follicular growth continued. This dose was sustained until the day hCG could be administered. No luteal support was provided.

Data analysis: Before initiation of the study, power calculations were performed to determine the required number of patients for the detection of differences in serum FSH and E_2 concentrations and number of dominant follicles on the day of hCG administration, comparing both dose regimens. Based on the literature and our previous studies (Schoot *et al.*, 1995; van Dessel *et al.*, 1995) differences were estimated to be 30%. This difference was calculated to be apparent with at least 12 patients in each group.

Values given are mean ± SD unless stated otherwise. The *P* values given are two-sided, and 0.05 was considered the limit of statistical significance. Differences between patient groups were tested using the Mann–Whitney test, Student's *t*-test, or Fisher's exact test. The distribution of ovarian follicles was arbitrarily classified in categories according to size (10–12, 12–16, and ≥16 mm), as described previously (van Santbrink *et al.*, 1995b). Ovulation after hCG administration was determined by the collapse of the dominant follicle on TVS and mid-luteal P concentrations. Pregnancy diagnosis was confirmed by a positive urinary pregnancy test (hCG, >25 IU/L), and ongoing pregnancy was confirmed by sonographic evidence of an intrauterine gestational sac and fetal heart beat. Patients attending our clinic within 2 weeks after the last administration of gonadotropins with serious abdominal discomfort, sonographic evidence of grossly enlarged ovaries, and increased quantities of free abdominal fluid, were reported to be suffering from ovarian hyperstimulation syndrome.

4.3.3 Results

Thirty-seven normogonadotropic patients with clomiphene-resistant anovulation entered the study protocol. After randomization, 19 (51%) patients received exogenous FSH treatment according to a low-dose step-up regimen, and 18 patients received a step-down dose regimen. Patient characteristics of both groups are shown in Table 7. The groups did not differ in any of the characteristics shown.

Table 7 Initial patient characteristics (median and range) of 37 normogonadotropic clomiphene-resistant oligo- or amenorrheic infertile women treated with urinary FSH for ovulation induction. Patients were randomized for low-dose step-up or a step-down dose regimens.

	Low-dose step-up	*Step-down*
n	19	18
Age (yrs)	28 (20–35)	30 (23–40)
BMI (kg/m^2)	26 (19–40)	25 (18–41)
Infertility duration (yrs)	3 (2–5)	3 (2–7)
Amenorrhea (%)	26	33
Primary infertility (%)[a]	63	61
Serum hormone concentrations[b]		
LH (IU/L)	5.6 (1.5–15.4)	5.8 (2.7–13.8)
FSH (IU/L)	4.9 (2.7–9.0)	4.5 (2.2–6.8)
E_2 (pmol/L)	238 (97–378)	250 (75–864)
T (nmol/L)	2.1 (1.3–4.6)	2.2 (1.4–4.3)
AD (nmol/L)	15.4 (8.5–30.4)	13.3 (9.3–32.8)
SHBG (nmol/L)	32 (10–128)	47 (20–164)
Transvaginal ultrasonography:		
Mean ovarian volume (mL)	8.5 (4.0–14.9)	11.3 (5.7–18.4)
Mean follicle number per ovary	12.5 (1.5–15.0)	14.3 (5.5–15.0)
Total stroma count[c]	4 (2–6)	4 (2–5)

[a]Absence of previous pregnancies in the present relationship >1 year.
[b]Blood samples taken at random in month preceding the study cycle.
[c]Scored on a scale from 1 (normal) to 3 (severely increased) and added for both ovaries.

In both groups one patient dropped out early due to extensive vaginal bleeding during gonadotropin treatment. Cancellation due to ovarian hyperresponse (more than 3 follicles ≥16 mm in diameter on the day of hCG administration) did occur once in the low-dose step-up regimen. Seventeen patients in both groups received hCG. One patient in each group presented with low luteal P concentrations (< 10 nmol/L), suggesting absence of ovulation.

Clinical treatment outcome is presented in Table 8. The median first day of ovarian response monitored by TVS in the low-dose step-up group was treatment day 11 (range, 5 to 33) *vs.* day 5 (range, 3 to 13) in the step-down group ($P<0.001$). The median day of administration of hCG in the low-dose step-up group was treatment day 20 (range, 8 to 42) *vs.* 10 (range, 5 to 17) in the step-down group ($P=0.001$). In the low-dose step-up group, 7 patients (39%) achieved a preovulatory follicle using not more than 1 ampule/day of urinary FSH. In the step-down group, the initial

Table 8 Clinical outcome of 35 normogonadotropic clomiphene-resistant oligo- or amenorrheic infertile women treated with urinary FSH for ovulation induction. Patients were randomized for a low dose step-up ($n = 18$) or a step-down dose regimen ($n = 17$).

	Low-dose step-up	*Step-down*	*P=*
Median duration of treatment (days)	18 (7–41)	9 (4–16)	0.003
Median # of ampules used per cycle	20 (7–69)	14 (7–33)	NS
Daily FSH change (IU/L) from the day of ovarian response until hCG.	+0.6 (+2%/day)	−1.3 (−5%/day)	<0.001
Patients with decreasing late-follicular phase FSH levels	6 (39%)	17 (100%)	<0.001
Mono-follicle growth[a] ($n=$)	10 (56%)	15 (88%)	0.04
Patients with E_2 within normal pre-ovulatory range (500–1500 pmol/L)	6 (33%)	12 (71%)	0.03
Cycles cancelled ($n=$)	2	1	
Overall ovulation rate	84%	89%	
Ongoing pregnancies ($n=$)	2	5	
Multiple pregnancies ($n=$)	0	0	
Abortion ($n=$)	1	0	
Tubal pregnancy ($n=$)	1	0	
Ovarian hyperstimulation ($n=$)	0	0	

[a]Defined as 1 follicle ≥16 mm on the day of hCG administration.

dose of 2 ampules/day was sufficient to induce ovarian response within 5 days in 14 (78%) patients.

Median serum FSH concentrations on the day treatment was started did not differ in both groups, and were higher on the first day of ovarian response, as determined by ultrasound, in the step-down group (6.3 IU/L [range, 4.7 to 10.2 IU/L] *vs.* 5.4 IU/L [range, 3.4 to 9.5 IU/L]; $P = 0.04$; Figure 12). From the day of ovarian response until the day of hCG administration, FSH concentrations showed an increase in 11 (61%) cycles in the low dose step-up group, whereas in the step-down group, FSH concentrations showed a decrease in all cycles ($P < 0.001$). The low dose step-up group exhibited a median increase in serum FSH during the late follicular phase of 0.6 IU/L (range, 2.0 to −4.3 IU/L) per day (=2% increase per day), whereas in the step-down group, FSH concentrations decreased during the same period (1.3 IU/L [range, 0.2 to 5.0 IU/L] per day [=5% decrease per day; Figure 12]). Differences between the groups in changes in serum FSH during the late follicular phase were highly significant ($P < 0.001$). Patients treated according to the low-dose step-up protocol with decreasing late follicular phase serum FSH concentrations exhibited monofollicle growth in 5 (71%) cases, whereas in the group with

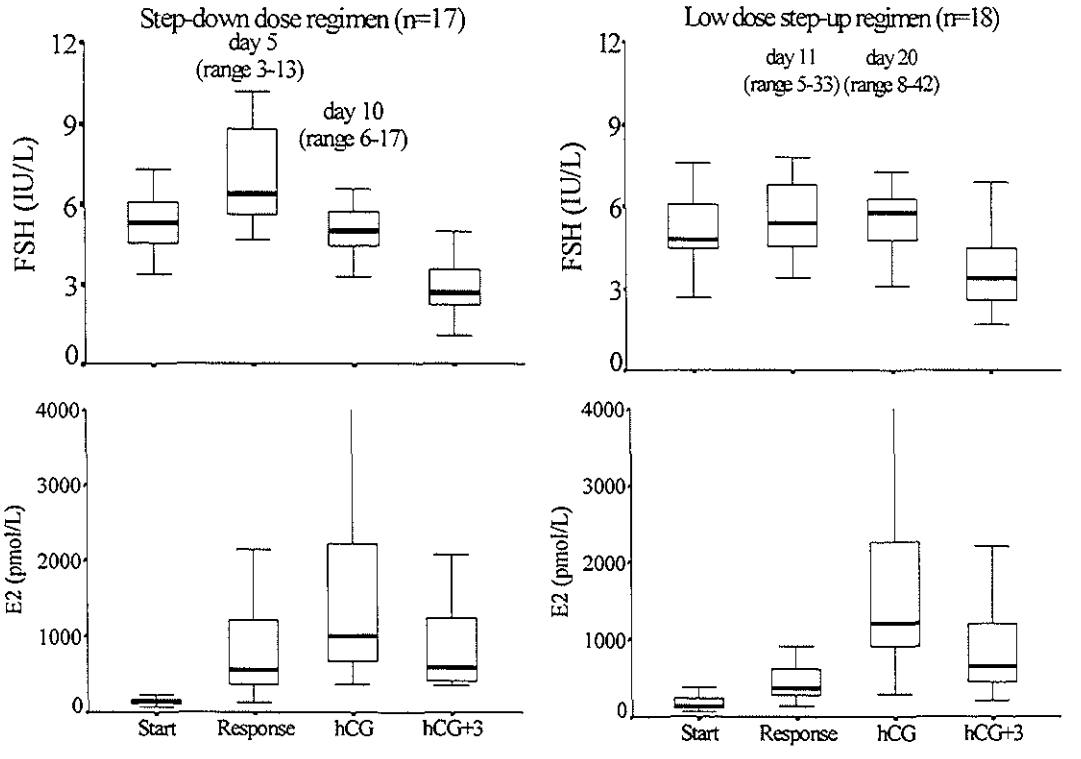

Figure 12 Serum FSH (IU/L) and E_2 (pmol/L) levels on the day of initiation of ovulation induction with urinary FSH (Start), the first day of ovarian response defined as the first day a dominant follicle can be recognized using transvaginal sonography (Response), the day of hCG administration to induce ovulation (hCG) and 3 days thereafter (hCG+3) in 35 normogonadotropic clomiphene- resistant oligo- or amenorrheic infertile women. This group was randomized for a step-down dose regimen (left panel) or a low-dose step-up regimen (right panel). Only cycles with ongoing follicle growth are included. Data are presented as box and whisker plots where boxes encompass values between the 25th and 75th percentile, horizontal lines represent median values, and whiskers give the 95% range of the values. See text for statistical evaluation.

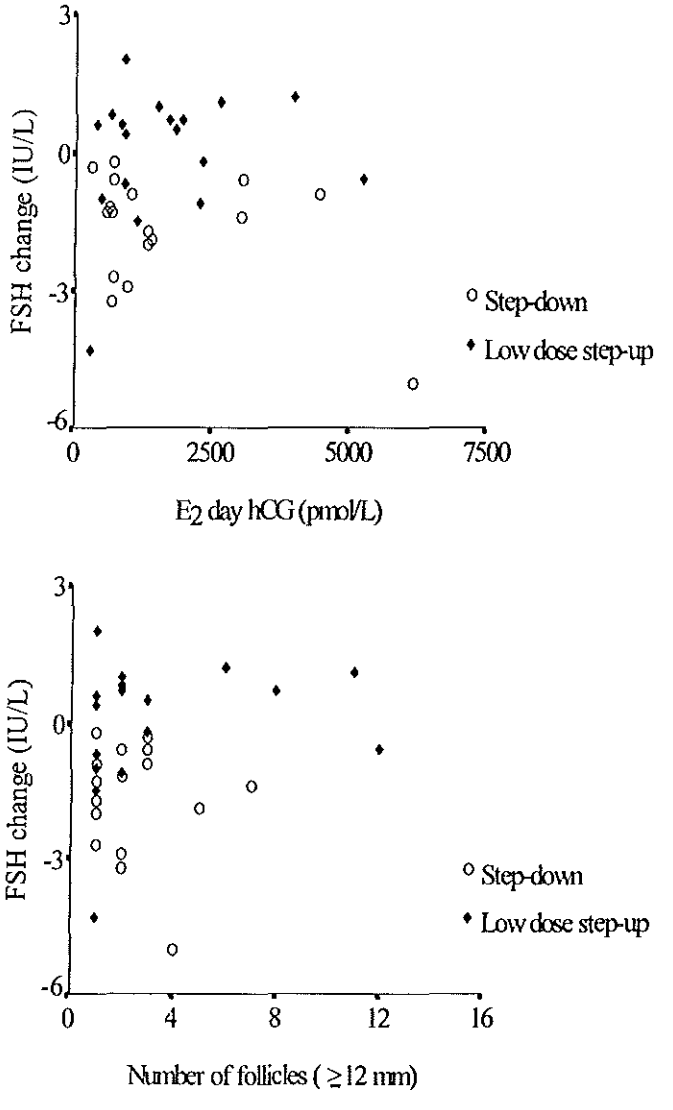

Figure 13 Distribution of serum E_2 levels (upper panel) and number of follicles ≥12 mm diameter (lower panel) on the day of hCG administration in a group of 35 normogonadotropic clomiphene-resistant oligomenorrheic or amenorrheic infertile patients treated after randomization according to a low-dose step-up (♦; n = 18) or a step-down dose regimen (o; n = 17) for induction of ovulation. Significant correlation could not be observed between serum FSH change in the late follicular phase (between the day of ovarian response and the day of hCG administration) and the above-mentioned parameters in the total study group or in both subgroups.

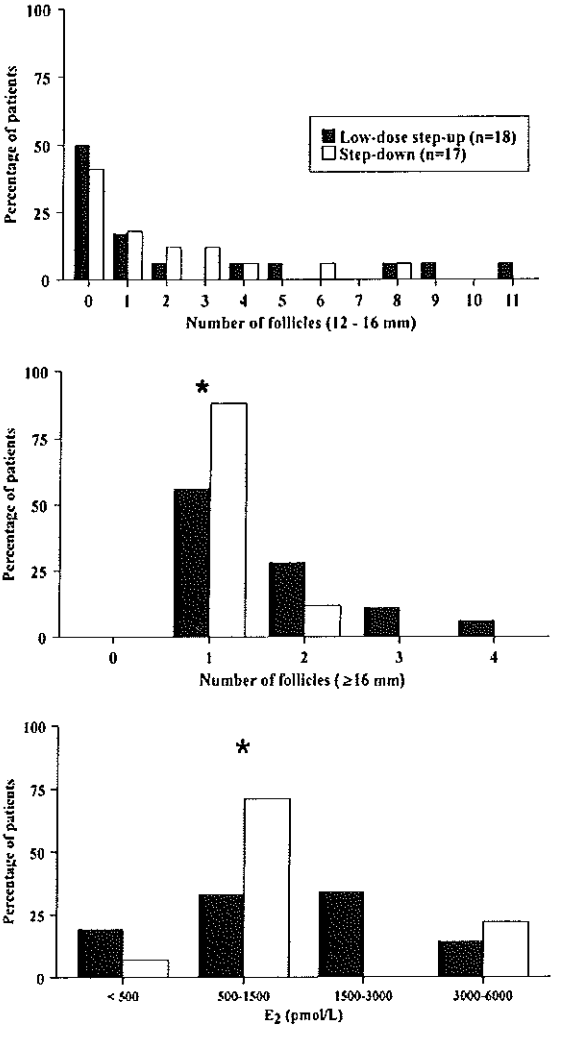

Figure 14 Distribution of number of ovarian follicles (arbitrarily classified as 12–16 mm diameter [upper panel], and ≥16 mm [middle panel) and serum E_2 levels (lower panel) on the day of hCG administration in 35 normogonadotropic clomiphene-resistant oligo- or amenorrheic infertile women randomized for a step-down dose regimen (n = 17) or a low-dose step-up regimen (n = 18) for induction of ovulation with urinary FSH. The asterisk indicates the significant difference (P = 0.04) in percentage of patients (56% vs. 88%) presenting with monofollicular cycles (1 follicle ≥16 mm only) comparing both treatment schedules. The percentage of patients (33% vs. 71%) with normal pre-ovulatory serum E_2 levels (500–1500 pmol/L) is also significantly (P = 0.03) different.

increasing late follicular phase serum FSH concentrations, there were 5 (45%) patients exhibiting monofollicular growth (P = NS). No significant correlation was observed between absolute individual changes in serum FSH concentrations in the late follicular phase (between the day of ovarian response and the day of hCG administration) and serum E_2 concentrations or number of follicles (≥12 mm in diameter) on the day of hCG administration in the total study group or in both subgroups (Figure 13).

The distribution of follicles (arbitrarily classified in size categories of 12–16 and ≥16 mm in diameter) on the day of hCG administration is shown in Figure 14. Medium-sized follicles of 10–16 mm in diameter were absent in 22% and 29% of low-dose step-up and step-down cycles, respectively (P = NS). Ovarian volumes on the day of hCG administration and 3 days thereafter showed no differences between both groups (data not shown).

The median increase in serum E_2 concentrations from the day of ovarian response until day of hCG administration was 179 pmol/L (range, 33 to 742 pmol/L) per day in the low-dose step-up group and 250 pmol/L (range, 23 to 1273 pmol/L) per day in the step-down group (P = NS).

In the step-up group, one pregnancy ended in an early miscarriage at 6 weeks, and one appeared to be a tubal pregnancy which had to be removed by laparoscopy. The ongoing pregnancy rate in the step-up group was 13%, compared with 31% in the step-down group.

4.3.4 Discussion

Knowledge regarding the interplay between serum FSH concentrations and follicle growth during the normal menstrual cycle has increased, and the process of selection of a single dominant follicle has been recognized to be dependent on decreasing FSH concentrations during the late-follicular phase (Zeleznik *et al.*, 1985; Lolis *et al.*, 1995). In contrast, increasing serum FSH concentrations in the late follicular phase have been described during conventional step-up regimens for induction of ovulation, even using a low-dose step-up regimen (Dale *et al.*, 1993). Step-up regimens ignore the concept that the FSH-threshold should be surpassed for a limited period of time only (a narrow window), sufficient to allow a single follicle to gain dominance (Brown *et al.*, 1978; Fauser *et al.*, 1997a). As complications of gonadotropin induction of ovulation, such as ovarian hyperstimulation and multiple pregnancy, are related to multiple follicle development (Blankstein *et al.*, 1987), it may be worthwhile to focus on various dose regimens and resulting patterns in serum FSH concentrations. In some ovulation induction studies (Dale *et al.*, 1993), late follicular phase steady state serum FSH concentrations were observed during low-dose step-up regimens. Moreover, preliminary data suggest that decreasing

serum FSH concentrations may occur due to negative estrogen feedback action in some patients (White *et al.*, 1995) or i.v. low-dose step-up FSH administration (van der Meer *et al.*, 1996).

Our group has focused on a method of obtaining serum FSH concentrations during gonadotropin induction of ovulation that more closely mimic physiology (Fauser, 1994). These studies have shown that the late follicular phase serum FSH profile during a step-down dose regimen with initial doses of 2 ampules per day and decreasing steps of a ½ ampule per day closely resembles FSH serum concentrations during the spontaneous cycle (Schoot *et al.*, 1995; van Dessel *et al.*, 1995), and that treatment outcome is at least comparable to that using low-dose step-up protocols (van Santbrink *et al.*, 1995b). To substantiate potential differences, we compared the step-down induction regimen with a low-dose step-up protocol in a prospective randomized trial. The results obtained indicate that in the step-down group, serum FSH concentrations decrease in the late follicular phase in all patients (median 5% per day), whereas in the low-dose step-up group, individual serum FSH concentrations decrease in only 39% of patients ($P<0.001$). The small number of patients (39%) in the low-dose step-up group that present with decreasing late follicular phase serum FSH concentrations consists of patients that in the majority of cases (71%) exhibit monofollicle growth; the 'good-responders'. Consistent with pharmacokinetic studies (Mizunuma *et al.*, 1991; Mannaerts *et al.*, 1993), the overall median serum FSH concentration in the low-dose step-up group remains fairly stable in the late follicular phase. Another study in which exogenous FSH was replaced by pulsatile administration of gonadotropin releasing hormone (GnRH) when dominant follicle growth was first recognized showed that multiple follicle growth was minimized, and a low multiple pregnancy rate could be obtained (Kuwahara *et al.*, 1995). The decrease in serum FSH during step-down ovulation induction in a protocol that combined exogenous gonadotropins with GnRH agonists showed a more pronounced median decrease of 10% per day (Schoot *et al.*, 1995) compared to the 5% daily decrease demonstrated in the present study. In a study in which patients were treated according to a low-dose step-up protocol using i.v. gonadotropin administration with and without pituitary down-regulation by GnRH agonists (van der Meer *et al.*, 1996), the effect was reversed; in the group treated with GnRH agonists, FSH serum concentrations were stable, whereas a minimal decrease occurred in the group treated with gonadotropins alone. This may be related to the changes in endogenous FSH production in non-suppressed patients. All of the above-mentioned observations favor a limited role for E_2 in late follicular FSH patterns in gonadotropin-stimulated cycles.

We observed more monofollicle cycles (88% vs. 56%) and more preovulatory E_2 serum concentrations in the physiological range (71% vs. 33%) in the step-down group than in the low-dose step-up group. This may coincide with reduced chances for multiple pregnancy and ovarian hyperstimulation (Haning *et al.*, 1983; Blankstein *et al.*, 1987). The reported percentages of monofollicle cycles in low-dose step-up studies vary substantially due to different criteria used for defining monofollicular growth and cycle cancellation. The small subgroup of patients in the step-down protocol with very high late follicular phase serum E_2 concentrations (patients that hyperstimulate with the starting dose) may respond better on a lower starting dose of gonadotropins. Another feature observed in this study is that the step-down group ovulated after a shorter induction time (9 vs. 18 days). Using a low-dose step-up protocol, patients may initially be exposed to serum FSH concentrations below the threshold for an extended period of time, resulting in a longer treatment period than strictly necessary. The treatment duration and number of ampules used in the low-dose step-up regimen in our study are comparable to data reported previously (Buvat *et al.*, 1989; White *et al.*, 1996). Few other studies have reported the application of exogenous FSH in a step-down fashion for induction of ovulation (Mizunuma *et al.*, 1991; Steinkampf *et al.*, 1993). The inclusion criteria used and a rigid step-down protocol render it difficult to draw conclusions from these studies. A large randomized study comparing conventional step-up and step-down regimens (initial dose 3 ampules/day) has only appeared in abstract form (Steinkampf *et al.*, 1993).

In conclusion, our findings suggest that in a group of normogonadotropic clomiphene-resistant anovulatory infertile women, induction of ovulation using a step-down gonadotropin dose regimen results in comparable ovulation and pregnancy rates and a much shorter induction period than that required with a low-dose step-up regimen. This may bring health economic benefits (more ovulations per given period of time). In addition, the late-follicular phase serum FSH profile is more physiological and results in more monofollicular cycles and more cycles in which E_2 concentrations are within the normal preovulatory range. These observations may have important implications for the risk of ovarian hyperstimulation and multiple pregnancy.

5

General Discussion and Conclusions

The first objective of this thesis was to evaluate the role which FSH plays in the process of follicle recruitment and subsequent selection of a single dominant follicle. We studied this issue during the normal menstrual cycle in healthy volunteers and in anovulatory infertile patients during gonadotropin induction of ovulation using a decremental dose regimen by frequent monitoring of follicle growth with the use of transvaginal sonography (TVS) in combination with serum blood sampling.

In a group of 16 strictly selected normal weight volunteers with regular menstrual cycles a major individual difference in FSH-threshold (2½-fold) for stimulation of follicle development was observed (see also Schipper *et al.*, 1998a). As maximum early follicular-phase FSH serum concentrations or FSH-decrease did not correlate with any patient or cycle characteristic, this finding may suggest major interindividual differences in FSH threshold. Variation may suggest individual differences in autocrine or paracrine modulation of FSH action during the process of follicle recruitment. During the late follicular phase, decreasing serum FSH concentrations in combination with an increasing sensitivity of the dominant follicle for FSH stimulation, probably modulated by intra-ovarian factors, facilitates the process of selection of a single dominant follicle (Schipper *et al.*, 1998b). Within the cohort of follicles, recruited by early follicular-phase rise in serum FSH concentration, each follicle has its individual developmental stage. The developmental stage determines the amount of FSH stimulation needed to prevent atresia, the FSH threshold. When FSH serum concentrations are decreasing, less mature follicles will go into atresia, while a single dominant follicle will continue its growth. We observed an association between the magnitude of decrease in early follicular phase endogenous serum FSH concentrations and the following E_2 serum rise, indicating that the duration of FSH stimulation is a major determinant for intra-ovarian granulosa cell aromatase activity, resulting in increased E_2 production when FSH stimulation is prolonged. A strong correlation between the first day a dominant follicle could be determined by sonog-

raphy and the start of a rise in serum E_2 concentrations indicates that transvaginal sonography is an accurate tool to monitor follicle development.

The patient group suffering from anovulatory infertility can be divided into a group with clear disturbances in gonadotropin serum concentrations (hypogonadotropic or hypergonadotropic amenorrhea) and a group with normal gonadotropins (normogonadotropic amenorrhea). A substantial part of patients in the latter group is described as polycystic ovary syndrome (PCOS). Because a clear controversy exists in the literature regarding endocrine and sonographic criteria used to define PCOS, we started to evaluate and compare criteria most frequently used in literature.

A more consistent definition of inclusion criteria used to determine PCOS may lead to better understanding of this heterogeneous entity. In addition, standardized initial screening of normogonadotropic anovulatory infertile patients – regardless of whether the criteria fit PCOS diagnosis – may help to predict outcome of ovulation induction (medication and doses needed, chances for success or complications) and long-term health risks. A study by our group (Imani *et al.*, 1998) has indicated that it may be possible to predict treatment response, during clomiphene-citrate ovulation induction in anovulatory normogonadotropic infertility, by initial screening of patient characteristics and cycle history; this approach will be the focus of a subsequent thesis from our group.

We observed considerable overlap between various parameters used in the literature to define PCOS and concluded that, after defining strict cut-off levels for sonographic and endocrine parameters in a control population, polycystic ovaries on ultrasound suffered from poor predictive value for endocrine abnormalities (hyperandrogenicity and/or elevated LH serum concentrations) used to define PCOS. So after all, the polycystic sonographic image of the ovaries may not be a very important parameter in anovulatory infertility. Another important issue is identification of initial patient characteristics, that can predict treatment response to gonadotropin induction of ovulation. This may help to further classify anovulatory infertile women and add to the ongoing discussion regarding criteria used to define PCOS. Accordingly, initial clinical, endocrine and sonographic characteristics were evaluated in a group of normogonadotropic amenorrheic infertile women with clomiphene-citrate resistance or failure treated according to a step-down dose regimen for induction of ovulation. Results of this preliminary study suggest that initial patient characteristics like obesity, ovarian aging (low basal E_2 serum concentrations and small initial ovarian volume), polycystic ovaries and elevated serum androgens (DHEAS and FAI) may be associated with impaired treatment response.

Future prospective, follow-up studies (Imani *et al.*, in preparation) will have to prove whether this approach is clinically useful.

Complications of induction of ovulation using exogenous gonadotropins in the group of normogonadotropic clomiphene-resistant oligomenorrheic or amenorrheic infertile women are a cause of major concern. These complications – mainly ovarian hyperstimulation and multiple pregnancy – are both reported to be correlated with late follicular phase FSH accumulation (Ben Rafael *et al.*, 1986) and multifollicular development (Blankstein *et al.*, 1987). In an attempt to reduce these complications alternative gonadotropin treatment schedules have been developed. The conventional step-up regimen has been modified to a 'low-dose' step-up regimen, which resulted in a decrease in complication rates but in some studies at the cost of lower pregnancy rates and an extended period of stimulation was necessary (Hull, 1991). Our group developed a new induction schedule trying to mimic physiological follicular phase serum FSH concentrations: the step-down dose regimen. Results indicated a more physiologic FSH serum profile, but a wide range in interpatient variability was observed. The protocol was modified to a low-dose step-down protocol (each step-down with ½ ampule/day) and a starting dose of 2 ampules/day. The first step-down was based on the identification of a dominant follicle by sonography and the minimum dose was 1 ampule/day. GnRH agonist pretreatment was abandoned because expected normal range serum E_2 concentrations would not result in premature luteinization and interference with endogenous gonadotropin release did not seem to have clinical implications. We conducted a clinical experience trial to test the step-down dose regimen for induction of ovulation as a safe and effective treatment alternative. This could be confirmed in a study in which a group of normogonadotropic clomiphene-resistant oligomenorrheic or amenorrheic infertile women was treated. The new step-down dose regimen appeared to be a clinically safe and effective treatment alternative (a much shorter induction period was needed and comparable ovulation and pregnancy rates were established).

A second step in the trial was to compare the step-down dose regimen to a low-dose step-up regimen for induction of ovulation in a randomized prospective study. This is the first reported randomized comparison between two different regimens for gonadotropin induction of ovulation. The low-dose step-up regimen is the most generally used gonadotropin induction schedule for ovulation induction. The comparison between the step-down and a low-dose step-up dose regimen in a group of normogonadotropic oligo- or amenorrheic clomiphene-resistant infertile women resulted in a significantly shorter induction period in the step-down group, while ovulation and pregnancy rates were at least comparable. In addition,

late-follicular phase FSH serum profile in the step-down group resembled physiology more closely, resulting in more monofollicular cycles and more cycles with serum E_2 concentrations within the pre-ovulatory range. In contrast, steady-state follicular phase serum FSH concentrations were observed during low-dose step-up protocols. These observations may have implications for chances of ovarian hyperstimulation and multiple pregnancies. A comparative multi-center trial to study the clinical outcome following both regimens is conducted in France at the moment. Although the step-down dose regimen may seem a new treatment alternative for all patients treated with exogenous gonadotropins for induction of ovulation, one should be cautious with direct clinical introduction. The following reasons should be considered: (1) for about 30% of all patients a fixed gonadotropin dose of 1 ampule/day is sufficient to stimulate dominant follicle development; (2) intensive monitoring is recommended (every 2–3 days) because there are hyper-responders on a 2 ampules/day starting dose; (3) monitoring a step-down dose regimen induction cycle requires more experience and skills. Therefore, step-down regimens potentially give rise to higher chances for complications in inexperienced hands.

Combining these study results into a safe and effective protocol to treat a new patient using exogenous gonadotropins for induction of ovulation, could be an initial 'dose-finding' cycle according to a low-dose step-up protocol. In this way patients exhibiting ongoing follicle growth using a starting dose of 1 ampule/day – the 'good responders'– can be identified. In patients not responding with ongoing follicle growth the daily dose should be increased. The second cycle is initiated according to a step-down dose regimen in these patients with a starting dose ½ ampule above the effective dose in the preceding low-dose step-up cycle. If no step-up has been necessary (the patient requires no more than 1 ampule/day to develop a pre-ovulatory follicle) the fixed dose regimen can be repeated. In this way, easy (low FSH threshold) and poor (high FSH threshold) responders are separated and complication rates may be lowered (In Preparation). It may also become possible, as discussed earlier, to predict whether a patient belongs to one group or the other, on the basis of initial screening characteristics (Imani *et al.*, In Preparation).

6

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7

Summary / Samenvatting

Chapter 1

This chapter aims at providing the reader with a brief overview of the history and development of knowledge regarding the function of the human ovary. It describes in more detail what is known about physiologic processes, regulating human follicle growth and selection of a single dominant follicle. Disturbances in follicle growth and possible treatment modalities in normogonadotropic clomiphene-resistant anovulatory women are discussed.

Chapter 2

To evaluate the role of FSH in follicle growth and selection of a single dominant follicle a group of 16 strictly regularly menstruating healthy women are studied. Daily blood samples are drawn and sonographic monitoring is performed every other day. Findings are a 2 ½-fold difference in early follicular maximum serum FSH concentrations indicating a wide range in FSH threshold between individuals. The first day a dominant follicle could be recognized by TVS was strongly correlated with the first day serum E_2 starts to rise. Because E_2 is produced mainly by the dominant follicle ultrasonography proves to be a reliable tool to monitor ovarian response. A negative association was observed between the decrease of serum FSH during the late follicular phase and the serum E_2 increase: FSH may induce E_2 biosynthesis in dominant follicle granulosa cells.

Chapter 3

Section 3.1 A lack of consensus on criteria used for PCOS diagnosis lead to this study in which we investigated the predictive value of polycystic ovaries diagnosed by TVS for endocrine signs of PCOS. First we set strict cut-off levels for endocrine and sonographic currently-used parameters in a control population. The use of these upper limits of normal in a group of 250 normogonadotropic oligomenorrheic or amenorrheic women visiting our fertility clinic resulted in 217 (66%) patients with polycystic ovaries

on ultrasound (defined as increased mean ovarian volume and/or mean follicle number per ovary), whereas 120 (36%) patients exhibited elevated serum androgens (increased serum AD and/or T) and 155 (47%) showed elevated LH concentrations. There was extensive overlap between these groups but limited predictive value of sonographic parameters for abnormal serum hormone concentrations.

Section 3.2 The objective of this study was to evaluate classification criteria in use for defining the normogonadotropic clomiphene-resistant oligomenorrheic or amenorrheic women with regard to their usefulness as predictor of treatment response in case of induction of ovulation using exogenous gonadotropins. Obesity, ovarian aging (low basal serum E_2 concentrations and small initial ovarian volume), polycystic ovaries and elevated serum androgens (DHEAS and FAI) were observed to be associated with impaired treatment response. More gonadotropins were needed and higher cancellation rates were observed.

Chapter 4

Section 4.1 Clinical experience with a step-down regimen for induction of ovulation with exogenous gonadotropins in a group of 82 normogonadotropic clomiphene-resistant anovulatory infertile patients is reported. In a total of 234 cycles a median treatment period of 11 days and a total of 14 ampules of gonadotropin were needed. Monofollicular growth was observed in 131 (62%) cycles while in 208 (98%) cycles not more than 2 follicles ≥16 mm diameter were present. A total of 37 pregnancies occurred of which 2 were twins and 1 was a triplet (multiple pregnancy rate: 8%). The pregnancy rate per cycle was 17% and the cumulative pregnancy rate after 7 months was 47%. The abortion rate was 19%. There were 4 cases of mild ovarian hyperstimulation, of which none became pregnant. The present study shows that the applied step-down regimen for gonadotropin induction of ovulation is a safe and effective treatment alternative for patients with clomiphene-resistant anovulation.

Section 4.2 To compare a low-dose step-up to a step-down regimen for induction of ovulation in a group of normogonadotropic clomiphene-resistant anovulatory infertile patients, this prospective randomized study was performed. The objectives were to assess potential differences in treatment duration and dose, ovarian stimulation (serum FSH concentrations) and response (serum E_2 concentrations and follicle number and size). Thirty-seven patients were included and randomized. The duration of the treatment period was reduced by 50% in the step-down group compared to the low-dose step-up group (9 vs. 18 days), the amount of gonadotropin

needed per cycle did not differ significantly (14 vs. 20 ampules). All patients in the step-down group showed a late-follicular FSH decrease compared with only 35% in the low-dose step-up group. Monofollicular growth on the day of hCG administration was observed in 88% of the step-down group versus 56% in the low-dose step-up group. Serum E_2 concentrations on the day of hCG administration were in the normal pre-ovulatory range in 71% of the patients in the step-down group while only in 33% of patients in the low-dose step-up group. In conclusion this study suggests that in a group of normogonadotropic clomiphene-resistant anovulatory infertile patients induction of ovulation according to a step-down dose regimen results in at least comparable ovulation and pregnancy rates while a much shorter induction period is needed compared to a low-dose step-up regimen. This may support health economic benefits (more ovulations per given period of time). In addition, late follicular phase FSH serum profile resembles physiology more closely resulting in more monofollicular cycles and more cycles with serum E_2 concentrations in the normal pre-ovulatory range. This observation may have important implications for chances of ovarian hyperstimulation and multiple pregnancy.

Chapter 5

This chapter aims at providing the reader with an overview of study results presented in this thesis, and these findings are discussed in the perspective of recent knowledge in this field including future perspectives.

SAMENVATTING (DUTCH)

Hoofdstuk 1

Dit hoofdstuk geeft een overzicht van de historie en ontwikkeling van kennis betreffende de functie van de menselijke eierstok. Fysiologische processen die een rol spelen bij follikelgroei en de selectie van de dominante follikel worden beschreven. Tevens worden stoornissen in follikelontwikkeling en mogelijkheden tot behandeling hiervan, met de nadruk op normogonadotrope clomifeen-resistente anovulatie, besproken.

Hoofdstuk 2

Om de rol van FSH ten aanzien van follikelgroei en selectie van de dominante follikel nader te onderzoeken wordt een groep van 16 gezonde strikt regulair menstruerende vrijwilligsters onderzocht. Er wordt dagelijks bloed afgenomen en om de dag transvaginale echografie verricht

gedurende een volledige cyclus. In de vroeg folliculaire fase wordt een 2½-voudig verschil gevonden in maximale FSH serum waarde. Dit geeft aan dat er een groot inter-individueel verschil bestaat in FSH drempelwaarde, die overschreden moet worden om follikelgroei te induceren. De eerste dag dat er echografisch een dominante follikel kan worden onderscheiden is sterk gecorreleerd met de eerste dag dat er een serum E_2 spiegel stijging plaatsvindt. Omdat het serum E_2 hoofdzakelijk door de dominante follikel wordt geproduceerd kan geconcludeerd worden dat echografie een betrouwbaar middel is om ovariele respons te monitoren. Er wordt een negatieve correlatie gevonden tussen de mate van FSH daling in de laat-folliculaire fase en de mate van E_2 stijging die hierop volgt. FSH lijkt direct de E_2-biosynthese te induceren in de dominante follikel, terwijl follikelgroei op dat moment steeds minder afhankelijk van FSH lijkt te worden.

Hoofdstuk 3

Paragraaf 3.1 Het polycysteus ovarium syndroom (PCOS) is een veel besproken maar slecht gedefinieerd ziektebeeld. Dit was de aanleiding om deze studie te verrichten, waarin wij trachten meer duidelijkheid te krijgen over de criteria die meestal in de literatuur worden gebruikt om PCOS te definieren, en in de mate waarin deze criteria onderling samenhangen. Er wordt gekeken of de mate van afwijking van echografische PCOS-parameters iets zegt over biochemische PCOS karakteristieken zoals beschreven in de literatuur. Hiertoe hebben wij eerst in een controle groep van strikt regelmatig menstruerende vrijwilligsters normaal waarden bepaald voor echografische en endocriene PCOS parameters. Deze normaalwaarden werden geprojecteerd op de studiegroep van 250 normogonadotrope infertiele patienten met een oligo- of amenorrhoea. Dit resulteerde in 217 (66%) patienten met echografisch polycysteuze ovaria (gedefinieerd als toegenomen gemiddeld ovarieel volume en/of toegenomen follikel aantal per ovarium), 120 (36%) patienten met verhoogde serum androgenen (toegenomen AD en/of T) en 155 (47%) patienten met verhoogde LH serum waarden. Er wordt uitgebreide onderlinge overlap tussen deze criteria gevonden, maar beperkte predictieve waarde van echografische parameters voor endocriene PCOS criteria.

Paragraaf 3.2 Het doel van deze studie is de classificatie criteria die voor onderverdeling van normogonadotrope clomifeen-resistente amenorrhoea worden gebruikt te evalueren voor wat betreft voorspellende waarde voor behandelingsrespons tijdens ovulatie-inductie met behulp van exogene gonadotrofinen. Overgewicht, ovariele veroudering (laag basaal E_2 en/of klein initieel ovarieel volume), polycysteuze ovaria en verhoogde serum

androgenen (DHEAS en FAI) zijn gecorreleerd met een verminderde behandelingsrespons. Er zijn meer ampullen gonadotrofine per cyclus nodig en een hoger aantal cycli moet voortijdig worden afgebroken.

Hoofdstuk 4

Paragraaf 4.1 Dit hoofdstuk behandelt de klinische ervaring met het 'step-down' doseringsschema voor ovulatie inductie, zoals in Rotterdam ontwikkeld. Een groep van 82 normogonadotrope clomifeen-resistente infertiele vrouwen werd gedurende de periode 1991 tot 1993 behandeld, met een totaal van 234 ovulatie-inductie cycli. De gemiddelde behandelingsduur was 11 dagen, waarin 14 ampullen gonadotrofine gebruikt werden. Er was een ovulatie aantoonbaar in 91% van de gestartte cycli en hiervan waren er 131 (62%) monofolliculair en 208 (98%) met niet meer dan 2 follikels ≥16 mm diameter op de dag van hCG toediening. Totaal kwamen er 37 zwangerschappen tot stand waarvan 2 tweeling en 1 drieling zwangerschap (meerling percentage 8%). Zwangerschaps percentage per cyclus was 17% en het cumulatieve percentage na 7 maanden was 47%. Abortus percentage was 19%. Er waren 4 gevallen van milde ovariele hyperstimulatie (zonder opname) waarvan niemand zwanger bleek te zijn. Deze studie toont aan dat het toegepaste step-down schema voor ovulatie inductie een veilig en effectief alternatief is voor patienten met een normogonadotrope clomifeen-resistente amenorrhoea.

Paragraaf 4.2 In deze gerandomiseerde studie wordt het step-down protocol voor ovulatie inductie met gonadotrofinen vergeleken met het low dose step-up protocol in een groep van patienten met een normogonadotrope clomifeen-resistente amenorrhoea. Het initiele studiedoel omvatte het vastleggen van potentiele verschillen in behandelingsduur en dosis, ovariele stimulatie (serum FSH spiegels) en ovariele respons (serum E_2 en follikelaantal en follikel grootte). Er worden 37 patienten geincludeerd en gerandomiseerd waarvan 18 in de step-down groep. De behandelingsduur is in de step-down groep gereduceerd met 50% vergeleken met de low dose step-up groep (9 dagen vs. 18 dagen), het aantal benodigde ampullen gonadotrofine verschilt niet significant (14 amp. vs. 20 amp.). In de step-down groep vertonen alle patienten een dalende FSH spiegel in de laat-folliculaire fase terwijl dit slechts in 35% van de patienten in de low dose step-up groep word gevonden. Serum E_2 spiegels op de dag van hCG gift zijn bij 71% van de patienten in de step-down groep binnen de normale pre-ovulatoire grenzen terwijl dit slechts bij 33% van de patienten in de low dose step-up groep kan worden vastgesteld. Monofolliculaire groei op de dag van hCG gift wordt bij 88% van de patienten in de step-down groep gezien terwijl dit in de low dose step-up groep in 56% van de gevallen

gevonden wordt. Concluderend kan over deze studie worden gezegd dat in een groep van patienten met een normogonadotrope clomifeen-resistente amenorrhoea, die wordt behandeld met ovulatie inductie middels exogene gonadotrofinen waarin een step-down en een low dose step-up protocol met elkaar wordt vergeleken, op zijn minst vergelijkbare ovulatie- en zwangerschaps percentages worden gevonden terwijl er een beduidend kortere inductieduur nodig is in de step-down groep. Dit kan duidelijk consequenties hebben op het gezondheids-economische vlak (meer ovulaties in een zelfde tijdsbestek). Verder laat deze studie zien dat het laat folliculaire FSH profiel in de step-down groep het fysiologische profiel beter benadert, hetgeen resulteert in meer cycli met E_2 serum spiegels in de normale pre-ovulatoire range en meer cycli met monofolliculaire groei. Dit kan belangrijke voordelen bieden ten aanzien van de kans op complicaties als ovariele hyperstimulatie en meerlingzwangerschappen.

Hoofdstuk 5

Dit hoofdstuk geeft een overzicht van de studieresultaten van dit proefschrift en probeert deze in het perspectief van de huidige kennis op dit gebied te plaatsen. Tevens wordt getracht om met deze resultaten in de hand een blik te werpen op toekomstige mogelijkheden.

8

Publications

List of publications included in the present thesis (numbers in bold refer to chapter sections)

2.1 van Santbrink, E.J.P., Hop, W.C., van Dessel, H.J.H.M., de Jong, F.H. and Fauser B.C.J.M. (1995) Decremental follicle-stimulating hormone and dominant follicle development during the normal menstrual cycle. *Fertil Steril* **64**, 37–43.

3.1 van Santbrink, E.J.P., Hop W.C. and Fauser, B.C.J.M. (1997) Classification of normogonadotropic anovulatory infertility: Polycystic ovaries diagnosed by ultrasound versus endocrine characteristics of polycystic ovary syndrome. *Fertil Steril* **67**, 453–458.

3.2 van Santbrink, E.J.P., Imani, B. and Fauser, B.C.J.M. (1997) Predictors of follicle development in gonadotropin induction of ovulation using a decremental dose regimen. In Filicori, M. and Flamigni, C. eds. *Ovulation Induction Update*. New York: Parthenon Publishing, pp. 67–74.

4.1 van Santbrink, E.J.P., Donderwinkel F.J., van Dessel, H.J.H.M. and Fauser B.C.J.M. (1995) Gonadotropin induction of ovulation using a step-down dose regimen: single-center clinical experience in 82 patients. *Hum Reprod* **10**, 1048–1053.

4.2 van Santbrink, E.J.P. and Fauser, B.C.J.M. (1997) Urinary follicle-stimulating hormone for normogonadotropic clomiphene-resistant anovulatory infertility: Prospective, randomized comparison between low-dose step-up and step-down dose regimens. *J Clin Endocrinol Metab* **82**, 3597–3602.

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Nilsson, C., Petterson, K., Millar, R.P., Coerver, K.A., Matzuk, M.M. and Huhtaniemi, I.T. (1997) Worldwide frequency of a common genetic variant of luteinizing hormone: an international collaborative research. *Fertil Steril* 67, 998–1004. (Evert J.P. van Santbrink and Bart C.J.M. Fauser, principal investigators in participating center).

Abstracts/presentations related to the present thesis:

Fauser, B.C.J.M., van Santbrink, E.J.P., van Dessel, H.J.H.M. and Hop, W.C. (1994) Decremental follicular phase FSH levels and follicle growth and selection in women with normal ovarian function. Abstract, Am. Fertil. Soc., 50th annual meeting, Nov. 5–10, 1994.

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Curriculum vitae auctoris

De schrijver van dit proefschrift, Evert Jan Pieter van Santbrink, werd geboren op 21 november 1962 te Rotterdam. De studie Geneeskunde werd in september 1981 begonnen aan de Rijks Universiteit van Utrecht. Het doctoraal examen werd in juli 1987 behaald en op 23 februari 1990 was het arts-examen een feit. Tijdens de co-assistentschappen werd de keuze voor de specialisatie Gynaecologie en Verloskunde steeds gemakkelijker (in tegenstelling tot de realisering hiervan). Van 1 april 1990 tot 1 april 1991 was eerdergenoemde werkzaam als arts-assistent Gynaecologie en Verloskunde in het Prot. Chr. Ziekenhuis 'de Lichtenberg' te Amersfoort. Van 1 april 1991 tot 1 januari 1993 was eerdergenoemde werkzaam als arts-assistent Gynaecologie en Verloskunde in het Westeinde Ziekenhuis te 's Gravenhage (opleider: Dr F.Th. J.G.Th. Kok / Dr P.J. Dörr). Hierna werd onder toeziend oog van Prof. Dr. B.C.J.M. Fauser de basis voor dit proefschrift gelegd van 1 januari 1993 tot 1 maart 1996, enerzijds als onderzoeker verbonden aan de Erasmus Universiteit Rotterdam en anderzijds als clinicus werkzaam op de polikliniek van de sector Voortplantingsgeneeskunde van de afdeling Verloskunde en Vrouwenziekten (hoofd: Prof. Dr B.C.J.M. Fauser). Vanaf 1 maart 1996 tot heden is promovendus werkzaam als arts-assistent Gynaecologie en Verloskunde in het St. Franciscus Gasthuis te Rotterdam (opleider: Dr A. Th. Alberda). Op 1 juni 1997 werd de opleiding tot Gynaecoloog gestart in het Rotterdamse opleidingscluster (opleider: Prof. Dr H.C.S. Wallenburg / Prof. Dr Th. J. M. Helmerhorst).

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