Prediction of Ovulation Induction Outcome in Normogonadotropic Anovulatory Infertility

Predictie van de ovulatie inductie resultaten in normogonadotrope anovulatoire infertiliteit

Prediction of Ovulation Induction Outcome in Normogonadotropic Anovulatory Infertility

Predictie van de ovulatie inductie resultaten in normogonadotrope anovulatoire infertiliteit

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam op gezag van de Rector Magnificus,

Prof.dr.ir. J.H. van Bemmel

en volgens besluit van het college voor promoties
de openbare verdediging zal plaatsvinden op
woensdag 6 november 2002 om 11.45 uur

door Babak Imani
geboren te Teheran, Iran

Promotiecommissie

Promotoren:	Prof.dr. B.C.J.M. Fauser
	Prof.dr.ir. J.D.F. Habbema
Overige leden:	Prof.dr. Th.J.M. Helmerhorst
	Prof.dr. E.R. te Velde
	Prof.dr. J. Collins

To
Dariush
My sunshine

The work presented in this thesis was performed at the Division of Reproductive Medicine (Head Prof. Dr. B.C.J.M. Fauser), Department of Obstetrics and Gynecology, Dijkzigt Academic Hospital and Erasmus University, Rotterdam, The Netherlands.

The author and studies presented in this thesis were supported by "Stichting Voortplantingsgeneeskunde Rotterdam" and an unrestricted research grant from Ferring Nederland BV

Most of this PhD manuscript has been published previously and has been reproduced with permission from the publishers:
Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (1998) Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, 83: 2361-2365
Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (1999) Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, 84: 1617-1622
Imani, B., Eijkemans, M. J., de Jong, F. H., Payne, N. N., Bouchard, P., Giudice, L. C., and Fauser, B. C. (2000) Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, 85: 676-682
Imani, B., Eijkemans, M. J., Faessen, G., Bouchard, P., Giudice, L. C., and Fauser, B. C. (2002) Prediction of the individual follicle-stimulating hormone threshold for gonadotrophin induction of ovulation in anovulatory infertility: An approach to minimize multiple gestation, ovarian hyperstimulation, and treatment expense. *Fertil Steril*, 77: 83-90
Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (2002) A nomogram to predict live birth probability after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *Fertil Steril*, 77: 91-97

Cover design: Darren Girotto, Shoreline Blvd, Mountain View 1675N, California, USA.
Cover: Antique original door knobs from an ancient port of a residential area of a village outside Yaz, Iran.
Typeset, printed and bounded by Historische Uitgeverij Rotterdam.
Organon Nederland BV, Ferring BV, and Serono Benelux BV are gratefully acknowledged for their financial support in the publication of this thesis.
First published 2002

ISBN: 90-73714-39-7

Contents

List of Abbreviations

AD	androstenedione
AUC	area under the curve
BMI	body mass index (weight / height^2)
CRA	clomiphene-citrate resistant anovulation
DHEAS	dehydroepiandrosterone sulphate
E_2	17b-estradiol
FAI	free androgen index (T x 100 / SHBG)
FSH	follicle-stimulating hormone
GnRH	gonadotropin releasing hormone
hCG	human chorionic hormone
HMG	human menopausal gonadotropin
IGF	insulin-like growth factor
IGFBP	IGF binding protein
im	intramuscular
IRMA	immunoradiometric assay
iv	intravenous
IVF	in-vitro fertilization
LH	luteinzing hormone
OHSS	ovarian hyperstimulation syndrome
P	progestrone
PCO	polycystic ovaries
PCOS	polycystic ovary syndrome
PFSH	purified urinary follicle-stimulating hormone
RIA	radioimmunoassay
ROC curve	receiver operating characteristic curve
sc	subcutaneous
SD	standard deviation
SHBG	sex-hormone binding globulin
T	testosterone
TSH	thyroid-stimulating hormone
TVS	transvaginal sonography
VEGF	vascular endothelial growth factor
WHO	World Health Organization

Chapter 1

Introduction and objectives

1.1 General introduction

Anovulation is a major cause of female reproductive dysfunction and can be identified in approximately 18-25% of couples presenting with infertility (Hull *et al.*, 1985). Oligomenorrhea (arbitrarily defined as menstrual periods occurring at intervals between 35 days to 6 months) or amenorrhea (menstruation intervals longer than 6 months) are common features. Whether and how frequent there occasional bleedings are associated with preceding ovulatory cycles is not known. The occurence of ovulation can be established applying basal body temperature charts, the assessment of serum progesterone levels, or through the observation by ultrasound of collaps of the pre-ovulatory follicle. While ovulatory cycles may be observed occasionally in oligomenorrheic women, ovulatory cycles are unlikely events in amenorrhea. Well-designed longitudinal follow-up studies concerning the incidence of spontaneous conception in oligomenorrheic patients are lacking. Follow-up studies performed thus far report only on pregnancies in those women who did not conceive spontaneously and subsequently seek the physician's help. Therefore, this population may be a negative selection of patients who exhibit cycle abnormalities. Obviously, induction of ovulation is required in these anovulatory patients to achieve follicular maturation, subsequent ovulation and ultimately conception.

The association between oligoamenorrhea, obesity, bilateral polycystic ovaries, and hirsutism was illustrated in 1935 by Stein and Leventhal (Stein *et al.*, 1935). A primary ovarian defect was inferred since bilateral wedge resection of the ovary restored the cycle abnormality unexpectedly and 2 of 7 patients conceived (Stein *et al.*, 1967). The wide variability of clinical and histologic findings associated with anovulatory state in PCOS resulted in the inability of the investigator to distinguish clinically significant and reliable characteristics of this syndrome (Goldzieher *et al.*, 1963). Excessive androgen production was initially attributed to abnormal adrenal function. Hyperandrogenemia due to diminished granulosa cell aromatase activity (responsible for the conversion of androgens to estrogens) of the polycystic ovaries (PCO) has subsequently been demonstrated (Axelrod *et al.*, 1962). Abnormalities in the hypothalamic-pituitary-ovarian axis resulting in inappropriate FSH secretion along with luteinizing hormone (LH) hypersecretion has also been highlighted (Yen *et al.*, 1970). Further insight in the abnormal physiology of this disorder occurred when hyperandrogenism was demonstrated to be LH dependent (Givens *et al.*, 1974). A landmark discovery was the association of ovarian hyperandrogenism and various causes of insulin resistance (Kahn *et al.*, 1976) and subsequently, an association between polycystic ovaries, hyperandrogenism and hyperinsulinemia was established (Burghen *et al.*, 1980).

The ultrasound manifestation of polycystic ovaries was first illustrated by Swanson and co-workers who demonstrated symmetrically enlarged ovaries containing numerous tiny cysts in various diameters which may be arranged in the periphery of an ovary (Swanson *et al.*, 1981). The ultrasonographic diagnosis of polycystic ovaries became accepted after diagnostic criteria had been critically defined (Forster *et al.*, 1988). Criteria for polycystic ovaries have – amongst many others - been investigated by our group applying transvaginal sonographic (TVS) assessment of the ovarian stroma echogenicity (arbitrarily classified from 1–3), ovarian volume (milliliters), and total number of follicles (both ovaries) (van Santbrink *et al.*, 1997b; Pache *et al.*, 1992). Polycystic ovaries has been defined as "mean ovarian volume ≥ 10.8 mL and/or mean follicle number per ovary ≥ 10" (van Santbrink *et al.*, 1997b).

However, criteria for PCOS diagnosis are not well defined. Various endocrine abnormalities such as hyperandrogenemia, hyperinsulinemia, or high LH have been used for the definition of PCOS (Fauser *et al.*, 1997). We studied the incidence of features associated with PCOS in normogonadotropic anovulatory infertility (see below), including high testosterone (T ≥ 3.2 nmol/L) and/or high androstenedione (AD ≥ 16.3 nmol/L) in combination with polycystic ovaries (van Santbrink *et al.*, 1997b).

1.1.1 Classification of anovulatory infertility

The clinical approach to ovulation induction in patients with ovarian dysfunction requires an understanding of the causes of anovulation. Classification of cycle abnormalities was originally described by Lunenfeld and Insler (Lunenfeld *et al.*, 1974) and has been subsequently modified by the World Health Organization (WHO) (Rowe *et al.*, 2001) and European Society for Human Reproduction and Embryology (The ESHRE Capri Workshop Group. *et al.*, 1995). According to this classification based on the assessment of gonadotropin and estrogen levels, patients are classified into three main categories (referred to as WHO classification; group 1, 2, or 3).

WHO class 1: Hypogonadotropic hypogonadal anovulation (hypothalamic-pituitary dysfunction): Women in this group, which accounts for 5-10% of anovulatory women, frequently present with amenorrhea. They have low or low-normal serum follicle-stimulating hormone (FSH) concentrations and negligible endogenous estrogen activity suggesting a central origin of the disease (i.e. decreased hypothalamic secretion of gonadotropin-releasing hormone (GnRH) or pituitary unresponsiveness to GnRH). The causes of hypothalamic amenorrhea include stress or exercise-related amenorrhea, anorexia nervosa, and Kallman's syndrome (isolated

gonadotropin deficiency). Approximately 5-10% exhibit hypopituitarism.

WHO class 2: Normogonadotropic normo-estrogenic anovulation (pituitary-ovarian 'dysbalance'): Women with normogonadotropic normoestrogenic anovulation constitute the largest group of anovulatory women encountered in clinical practice (60-85% of cases). These patients present with serum FSH (1-10 IU/L) and estradiol levels within the normal range (World Health Organization. *et al.*, 1993; Rowe *et al.*, 2001). The polycystic ovary syndrome (PCOS), characterized by chronic hyperandrogenic anovulation, comprise a significant proportion of these patients (20-25%) (Rowe *et al.*, 2001). It constitutes the most common cause of WHO class 2 anovulatory infertility. Strictly speaking, the term normogonadotropic does not apply to some women since approximately 50% present with elevated LH. WHO 2 (and to a lesser extent PCOS) represent a notoriously heterogeneous patients group, since different underlying causes might be involved which lead to a comparable clinical manifestation *i.e.* chronic anovulation.

WHO class 3: Hypergonadotropic hypoestrogenic anovulation: The patients in this group account for 10-30% of cases of anovulation. These individuals present with high levels of gonadotropins and low circulating endogenous estrogens levels suggesting the presence of a nonfunctioning ovary. The primary causes represent auto-immune disorders, genetic abnormalities or not yet elucidated factors that constitute conditions in which the number of the primordial follicles drastically diminish prior to the timed menopause (premature ovarian failure).

1.1.2 Incentive for development of a sub classification of WHO 2

Despite the extensive endeavor of investigators who have focused on the etiology of PCOS, no clear consensus has been reached concerning the validity of criteria used to diagnose this syndrome as well as its relevance for clinical practice (Franks *et al.*, 1995). We have previously demonstrated that a distinct overlap exists between three criteria used for polycystic ovary diagnosis based on ultrasound (van Santbrink *et al.*, 1997b). Moreover, these sonographic characteristics overlap the endocrine parameters such as LH, T or AD (Pache *et al.*, 1991; Pache *et al.*, 1993; van Santbrink *et al.*, 1997b). If strict criteria are used for the diagnosis of PCOS, a large heterogeneous group of WHO 2 patients will remain unclassified. All this confusion underlies a seemingly never-ending discussion regarding appropriate criteria for PCOS diagnosis. Controversy will continue as long as end-points are not defined.

It is generally accepted that PCOS patients represent a distinct subgroup with lower pregnancy chances and an increased risk for development of complications during ovulation induction such as ovarian hyperstimulation syndrome (OHSS),

multiple pregnancy, or miscarriages. However, reports in the literature are conflicting due to inclusion bias of population studied and the retrospective nature of most studies (Fauser *et al.*, 1997). Reflecting the lack of consensus in defining PCOS, various criteria are used for PCOS diagnosis. This obviously affects the inclusion of the remaining group of WHO 2 anovulatory infertility in the study. Some features of PCOS can be found in almost all WHO 2 patients and there is a gap in the literature regarding the behavior of these patients during ovulation induction.

In daily clinical practice, it does not seem to matter whether a woman is diagnosed as having PCOS, since all WHO 2 anovulatory patients start with first line anti-estrogen medication clomiphene citrate (CC). Altogether, less than 50% of women will conceive following CC and very little is known regarding the clinical, endocrine, and ultrasound characteristics of these patients. This becomes increasingly important with the current trend to delay childbearing, since time lost with ineffective therapy will by itself decrease chances for subsequent therapeutic strategies. Since, the primary wish of infertile couples is the live birth of a child, a reasonable approach may be to screen anovulatory infertile women with this endpoint in mind. Screening of anovulatory infertile women should be performed prior to initiation of ovulation induction with the following aims:

Assessment of health risks; Although extremely rare, anovulation may be the first sign of a serious underlying disease , such as ovarian, adrenal or pituitary tumors.

To assess whether ovulation induction is indicated. For instance, medical intervention to induce ovulation is useless in WHO 3 anovulation where ovarian function per se is defective due to exhaustion of the primordial follicle pool.

Choice of most appropriate type of ovulation induction agent; For instance, pulsatile GnRH in hypogonadotropic hypogonadism and CC in WHO group 2 anovulation. It is still uncertain whether the addition of dexamethasone in cases of adrenal hyperandrogenemia may increase chances of ovulation or conception. Further studies should also establish whether and for which patients insulin sensitizing agents are indicated.

Assessment of doses required; Currently, most clinicians start with the lowest possible gonadotropin dose (50 or 75 IU of FSH), and increase the dose slowly and prudently (so called, low-dose step-up protocols) to minimize the chance for OHSS. This is a low risk but time consuming approach. Were dose requirements known for a given patient, then treatment strategies could be individualized. This approach would increase efficiency and render this treatment modality more cost-effective. Starting with the individualized accurate dose (predicted FSH threshold or response dose) in decremental FSH dose regimens, however, would certainly improve safety.

Assessment of chances for success or complications; Patients with low chances may be directly shifted to alternative treatment options (such as exogenous gonadotropins as first line treatment, or IVF rather than ovulation induction). Patients with high chances for complications should be monitored more closely.

Assessment of chances of long-term health risks; For instance, increased risks of developing type 2 diabetes later in life on the basis of insulin resistance, or presumed cardio-vascular risks and chances for developing endometrial cancer in PCOS. Very few data are available in the current literature, and it is much too early to assess whether any prospective measure may improve life expectancy. Results from appropriately designed prospective follow-up studies are awaited with much interest.

1.2 Treatment strategies in WHO 2 anovulatory infertility

The anti-estrogen clomiphene citrate (CC) represents the first line treatment strategy in WHO class 2 anovulatory infertility. Medication is cheap and complications are minor. However, the majority of patients fail to conceive following CC therapy. Generally, these patients will subsequently be treated with daily administration of exogenous FSH. This treatment modality is associated with a higher ovulation and conception rate at the expense of a clear increase in the chance for complications such as multiple pregnancies or OHSS. The last step of treatment strategy is *in vitro* fertilization (IVF). A schematic presentation of the treatment strategies, ovulation induction protocols and possible treatment outcomes has been depicted in Figure 1. In this section we describe in brief the ovulation induction protocols used in daily clinical practice.

1.2.1 Anti-estrogens

Clomiphene citrate consists of two isomers with mixed estrogen and anti-estrogen activities (Adashi *et al.*, 1996). Many organs have estrogen receptors, and therefore CC may act at many sites in the body (Adashi *et al.*, 1996). Although rising serum FSH levels due to CC interference with estrogen negative feedback may be held responsible for stimulating follicle growth (Jacobson *et al.*, 1968; Miyake *et al.*, 1983; Kerin *et al.*, 1985), other mechanisms of action have also been proposed (Butzow *et al.*, 1995; Adashi *et al.*, 1996).

Generally, clomiphene is initiated between day 3 and day 5 after a spontaneous or progestagen-induced withdrawal bleeding. The starting dose is 50 mg/day, orally, for 5 subsequent days after initiation of menstruation. Daily doses are

increased by 50 mg in the subsequent cycle to increase the endogenous FSH rise and induce follicle maturation. The highest recommended CC dose is 150 mg/day which generally is used in the subsequent cycle after a given WHO 2 patient persist to remain anovulatory following 100 mg of CC. If ovulation occurs, the CC dose used should remain unaltered for subsequent cycles. Some publications proposed higher daily doses (Lobo *et al.*, 1982a) or an extended duration of treatment (Fluker *et al.*, 1996).

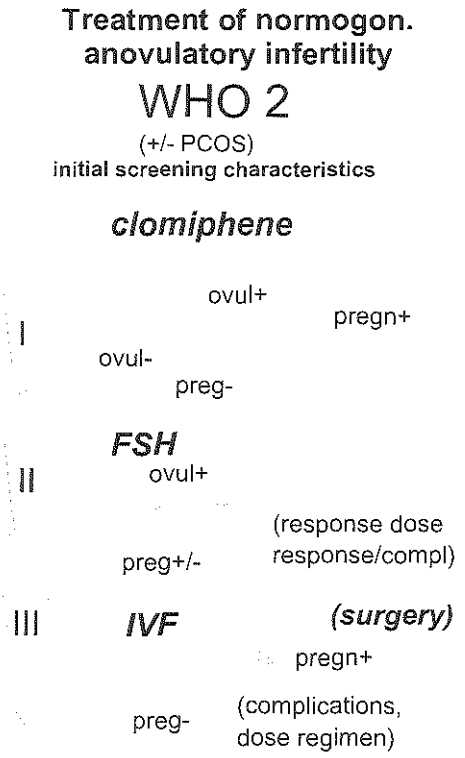

Figure 1.2. *Schematic representation of classical ovulation induction treatment modalities (and outcomes) in normogonadotropic anovulatory infertility.*

However, the additional benefit in terms of the number of live birth applying these alternative approaches may be negligible and should be investigated. There is not a common consensus regarding the duration of the CC treatment if a given WHO 2 patient's cycle abnormality is restored. However, ovarian response in these patients should be monitored in a regular basis i.e. every 3 months. A menstruation chart or a body temperature chart may be used to monitor the cycle pattern during CC use at home. Ovulation following CC can also be monitored by; (1) The

assessment of a midluteal serum progesterone (P) measurement (levels >25 nmol/L indicating ovulation). (2) Transvaginal sonographic monitoring of follicle growth until the appearance of a pre-ovulatory follicle (mean diameter, ≥18 mm) and subsequent follicle rupture after endogenous LH rise.

1.2.2 Exogenous gonadotropins

Since their introduction into clinical practice in 1958, exogenous gonadotropin preparations play a central role as the second line modality for ovulation induction in WHO 2 patients who fail to ovulate or conceive during the previous anti-estrogen medication (Schwartz *et al.*, 1981; Lunenfeld *et al.*, 1985; Insler *et al.*, 1988; Kelly *et al.*, 1990; Fauser *et al.*, 1993; Franks *et al.*, 1994; Fauser *et al.*, 1997). 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. Characteristics of dominant follicle development during gonadotropin induction have been monitored using ultrasound and serum estrogen levels (Wilson *et al.*, 1982; Marrs *et al.*, 1983; Messinis *et al.*, 1990; Schoot *et al.*, 1992).

As indicated previously, this treatment modality is associated with a higher chance for complications such as OHSS or multiple pregnancy. During the first decade of clinical use, various dose regimens, such as fixed, intermittent, or flexible incremental or decremental doses, have been tested (Taymor *et al.*, 1967; Thompson *et al.*, 1970). Recently, various novel protocols, new FSH preparations and tools have been introduced to improve ovarian response monitoring and minimize the chance of complication. These novel protocols will be discussed later in the present chapter;

1.2.3 Alternative treatment modalities

Recently introduced insulin sensitizing agents such as Metformin may be used as an alternative approach in (insulin resistant) anovulatory patients particularly in women remaining anovulatory following CC (Kocak *et al.*, 2002). More recently aromatase inhibitors have been introduced and may be used as ovulation induction medication in the future (Mitwally *et al.*, 1902). Laser surgery and ovarian drilling may be used to restore the cycle and endocrine abnormality of these patients although there is insufficient evidence to demonstrate a significant effect in ovulation or pregnancy rates (Farquhar *et al.*, 2000). *In vitro* fertilization is the last option in the treatment of WHO 2 anovulatory infertility. This is the most unknown area in terms of understanding ovarian response during treatment and

prediction of treatment outcome (complications and live birth) partly due to a lack of prospective follow-up studies. *In vitro* fertilization is certainly the most expensive strategy in terms of treatment costs per cycle. Diet and exercise may also be a different approach in restoring the ovarian abnormality and should be combined with most of the treatment strategies to increase the chance of spontaneous or stimulated ovulation and conception (Hollmann *et al.*, 1996; Clark *et al.*, 1998).

1.3 Success and complication rates of ovulation induction protocols

Approximately 70-80 % of WHO 2 anovulatory infertile women will ovulate after CC medication (MacGregor *et al.*, 1968; Gorlitsky *et al.*, 1978; Shepard *et al.*, 1979; Hammond *et al.*, 1983; Polson *et al.*, 1989; Opsahl *et al.*, 1996), whereas only 40-50% of ovulatory women will conceive (Gorlitsky *et al.*, 1978; Hammond *et al.*, 1983) following CC. However, CC remains the first line treatment strategy in normogonadotropic anovulatory patients, because it is simple and inexpensive with little chances for side-effects or complications. Means of identifying women who will remain anovulatory following CC therapy have, until recently, been restricted to clinical experience and retrospective analysis indicating obesity and hirsutism to be negative factors (Lobo *et al.*, 1982b).

Although gonadotropin therapy has been shown to be fairly successful in terms of ovulation rates (reported in the literature between 60-100%) (White *et al.*, 1996; Imani *et al.*, 1998) and cumulative pregnancy rates (reported between 20-75%) (White *et al.*, 1996; van Santbrink *et al.*, 1995; Imani *et al.*, 1999), complication rates are high (Fauser *et al.*, 1997). The most prominent complications include multiple pregnancies and OHSS.

The chance of success and complications in each treatment strategy varies. Relevant factors will now be discused separately;

1.3.1 Patients related factors

Variability in the FSH dose requirements due to distinct individual FSH threshold levels
Women diagnosed as hypogonadotropic hypogonadism, by definition, suffer from inadequate stimulation of ovarian function. FSH serum levels are below the threshold, and growth of follicles is arrested at a stage where further development becomes dependent on stimulation by gonadotropins. If FSH levels rise above the threshold, due to exogenous administration of gonadotropin preparations, ovarian response should be normal. Success and complication rates of gonadotropin induction of ovulation in these patients is certainly favorable

(Oelsner *et al.*, 1978; Dor *et al.*, 1980; Healy *et al.*, 1980; Schwartz *et al.*, 1980; Wang *et al.*, 1980; Ginsburg *et al.*, 1991)(Hull *et al.*, 1991).

The great majority of WHO 2 patients who remain anovulatory following CC medication are the potential candidates for induction of ovulation with exogenous gonadotropins. Major complications during gonadotropin ovulation induction include multiple pregnancies (Schenker *et al.*, 1981), ovarian hyperstimulation (Golan *et al.*, 1989), and a high rate of early pregnancy wastage (Shoham *et al.*, 1991b). In this regard, a profound individual variability in ovarian response to stimulation by FSH (so-called 'FSH threshold') was proposed in anovulatory patients indicating that only a small margin exists between an effective dose and a dose generating excessive ovarian response due to multiple follicle development (Brown *et al.*, 1978). Unfortunately, predictors for the FSH threshold of a given WHO class 2 patient have not yet been identified.

Various lines of evidence indicate that early follicle development is normal in WHO 2 patients particularly in PCOS, whereas anovulation is caused by disturbed dominant follicle selection (Pache *et al.*, 1992). This abnormal condition may be caused by disturbed intraovarian regulation of FSH action (Fauser *et al.*, 1994), and therefore response to exogenous FSH may be different from normal. Hence, the presence or absence of ovarian abnormalities in patients may influence treatment outcome after exogenously administered gonadotropins. This may explain major differences in the FSH threshold and duration of stimulation needed to induce preovulatory follicle development in these patients.

The FSH response dose (FSH threshold) may represent the severity of ovarian abnormalities in WHO 2 patients and may be associated with reproductive outcome such as live birth during gonadotropin ovulation induction. Major individual variability in FSH threshold and ovarian response to stimulation by exogenous FSH underscores the requirement for regular and cautious monitoring of ovarian response by ultrasound and/or rapid serum E_2 assays (Schoot *et al.*, 1992) and fine-tuning of exogenous FSH doses on an individual basis. Disparity in the FSH threshold level of WHO 2 individuals result in significant variability in the duration of gonadotropin administration. Unaltered late follicular phase FSH serum levels in gonadotropin-induced cycles differ significantly from the follicular phase of the normal menstrual cycle. This condition may elicit growth of other cohort follicles and consequently, induce multiple follicle development due to extension of the FSH window.

Oligomenorrheic patients might constitute a different subset of WHO 2 patients compared to amenorrheic subjects. Amenorrheic women may present with low or normal E_2 levels whereas estradiol in oligomenorrheic women is usually within normal range. There are several studies indicating that frequency of PCO

is different in amenorrheic compared to oligomenorrheic subjects (Devoto *et al.*, 1998; Hull *et al.*, 1987). Although LH pulse frequency and intervals are not different, the mean pulse amplitude is significantly higher in amenorrheic patients compared to oligomenorrheic subjects (Preleviç *et al.*, 1990).

Obesity frequently coincides with PCOS, and differences in pharmacokinetic characteristics of gonadotropin preparations (Mannaerts *et al.*, 1993), as well as clinical outcome (McClure *et al.*, 1992) related to body weight, have been reported. Moreover, other concomitant endocrine disorders such as hyperprolactemia or adrenal hyperandrogenemia may also affect treatment outcome.

Increasingly, the wish to establish a family is expressed later in life. Therefore, the population of women seeking help for infertility is increasing in age. It has been documented that cumulative conception rates after gonadotropin induction of ovulation are distinctly different when women under the age of 35 are compared with older women (Dor *et al.*, 1980; Ginsburg *et al.*, 1991).

1.3.2 Medication related factors

Several stimulation protocols have been introduced and applied in the clinic applying low-dose exogenous gonadotropins regimen to minimize the chance of complication. Conventional step-up dose regimens for gonadotropin induction of ovulation are characterized by initial daily doses of two ampoules (= 150 IU of bioactive FSH). Doses may be increased after 5 days in the event that ovarian response is judged to be insufficient. This protocol has been the preferred dose regimen worldwide since the early 1970s. Estimation of ovarian response changed over time from physical examination (urine and later serum) to estrogen assays, to abdominal ultrasound, and more recently TVS. Improved accuracy of response monitoring resulted in superior treatment outcome. This treatment modality is effective, with a relatively high complication rate (Thompson *et al.*, 1970; Oelsner *et al.*, 1978; Dor *et al.*, 1980; Healy *et al.*, 1980; Wang *et al.*, 1980). This is now believed to be related to FSH serum levels being too far above the threshold in a great proportion of patients. However, few studies have focused on FSH serum levels and ovarian response during conventional step-up cycles (Wu *et al.*, 1977; Healy *et al.*, 1983; Mallya *et al.*, 1993).

This dose regimen is characterized by low initial daily gonadotropin doses ranging between one-half and one ampoule (38-75 IU of bioactive FSH), and doses are only increased by one-half ampoule per day after 14 days, in cases of insufficient ovarian response (Seibel *et al.*, 1984). This treatment modality seems to be characterized by low complication rates, at the price of an extended duration of treatment and possibly a slightly diminished success rate (Seibel *et al.*, 1984; Buvat

et al., 1989; Hamilton-Fairley *et al.*, 1991; Shoham *et al.*, 1991a; Dale *et al.*, 1992; Cheng *et al.*, 1993; Buckler *et al.*, 1993; Dale *et al.*, 1993; Herman *et al.*, 1993; Balen *et al.*, 1994; Strowitzki *et al.*, 1994; Homburg *et al.*, 1995). The FSH threshold for stimulation of ovarian activity is reached slowly and prudently, in an attempt to reduce the magnitude of serum FSH levels surpassing the individual threshold (Ben *et al.*, 1995). This means that in a given patient, gonadotropins are administered for an extended period of time, due to the individual profound variability of FSH threshold level. The amount of exogenous FSH will be increased after 14 days of treatment in the event that a relatively high FSH threshold is operative. Pharmacokinetic studies have indicated that it takes approximately 5 days before steady state FSH levels are reached when similar gonadotropin doses are administered daily through the intraperitoneal route (Mannaerts *et al.*, 1993; Mizunuma *et al.*, 1990). Therefore patients may be exposed to FSH levels that are too low to stimulate follicle growth for several weeks. It is uncertain whether ovaries may be sensitized by extended exposure to subthreshold FSH levels. Similar daily serum FSH levels were measured preceding hCG administration in patients treated with low-dose, step-up protocols (Dale *et al.*, 1993). Hence, in the late follicular phase FSH serum levels remain above the threshold for an extended period of time, resulting in a wide FSH window even when low-dose step-up protocols are used. Improved treatment outcome, as compared with conventional step-up protocols, is likely due to the reduced magnitude of FSH levels surpassing the threshold when lower initial doses are used. Improved monitoring of ovarian response should not be ruled out as an additional important factor responsible for improved safety of treatment.

Our group has focused on establishing a protocol for gonadotropin induction of ovulation applying decremental doses once there is an onset of ovarian response following sonographic monitoring (so-called 'step-down' protocol) (Fauser *et al.*, 1993b). The major goal has been to design a safe and effective dose regimen for gonadotropin induction of ovulation that mimics physiological circumstances as closely as possible (Schoot *et al.*, 1992; Schoot *et al.*, 1993; Schoot *et al.*, 1995; van Dessel *et al.*, 1996). Readers are referred to the previous publications of our group for more detail (Schoot *et al.*, 1992; Schoot *et al.*, 1993; van Santbrink *et al.*, 1995; Schoot *et al.*, 1995; van Dessel *et al.*, 1996; Fauser *et al.*, 1997; van Santbrink *et al.*, 1997a). In brief, we applied the following FSH dose regimen; i.e. a two-ampoule/day starting dose shortly after a spontaneous or progestagen-induced bleeding, followed by a decrease to one and one-half ampoules/day once a dominant follicle can be visualized by TVS (at least one follicle > 10 mm) (van Santbrink *et al.*, 1995; Fauser *et al.*, 1997; van Santbrink *et al.*, 1997a). The dose is further decreased to one ampoule/day 3 days after the first dose reduction (van Santbrink *et al.*, 1995). The stimulation duration is approximately 10 days (medi-

an) and from 234 stimulated cycles in only one or two large preovulatory follicles were observed in 95% of stimulated cycles (van Santbrink *et al.*, 1995). As compared with step-up protocols, a significant reduction in late follicular phase E_2 levels and number of large and medium-sized follicles was observed in a randomized controlled comparative study performed by our group (van Santbrink *et al.*, 1997a). Moreover, the pregnancy chances were not reduced compared to those indicated following the low-dose step-up dose regimens. The observed reduction in the duration of stimulation, as well as a lower total number of ampoules per stimulation cycle, may represent significant benefits in terms of health economics (reduced drug costs per cycle, possibly a reduced number of visits to the clinic, and more ovulations in a given time period). The major problem of this approach is that the initial starting dose may be too high for a proportion of WHO 2 patients particularly those women with a low FSH threshold. For low-dose step-up regimen, however, the major problem might be the duration of ovarian stimulation which is extended in patients with an augmented FSH threshold who start with an initial low-dose exogenous FSH.

1.4 Statistical aspects of the development of a prognostic model

The clinical end point in a prospective study for prediction of infertility treatment outcome may be ovarian response to medication, conception or ongoing pregnancy leading to live birth. At the end of follow-up in a prospective fashion, some patients have not yet reached the end point. Therefore, statistical methods have been introduced to incorporate censoring, which are based on the assumption 'non-informative drop-outs". The fact that a patient does not continue the fertility therapy does not indicate her chance of becoming pregnant since the statistical formula used in these methods are based on the assumption that the chances of becoming pregnant in all patients who did or did not reach the end point are comparable (Kaplan *et al.*, 1958; Cox *et al.*, 1972). In this respect there is no problem with a given patient who voluntarily stops the fertility treatment. However, women who are advised to stop the fertility treatment because of the adverse ovarian response are informative drop-outs. An excellent example for this condition is patients who remain anovulatory following CC who are advised to stop the first line medication and start with an alternative treatment modality such as induction of ovulation using exogenous gonadotropins. For this reason we focused on distinct outcomes (ovulation *vs.* conception and life birth) in our studies focusing on prediction of CC outcome.

So far only univariate analyses have been used in the studies on prediction of fertility outcome in the literature which does not take into account the dependen-

cy between the candidate predictors. For this reason, multivariable analyses have recently been developed and used in the studies which have aimed to develop models for prediction of chances to conceive in normo-ovulatory sub/infertile women (Eimers *et al.*, 1994; Collins *et al.*, 1995). For practical use, the model may be visualized applying a score chart or a Nomogram. Finally, a prognostic model should be internally validated to correct the possible "overfitting". This is due to the development of a multivariate model with many candidate potential predictors on a limited number of patients reaching the end point.

1.5 Study objectives

It would potentially represent a significant step-forward in the development of an individualized treatment strategy if patients with a very low chance for live birth after CC could be predicted beforehand. Alternative treatment approaches may be applied in patients who have a high chance of remaining anovulatory following CC or despite ovulatory CC-induced cycles do not conceive leading to live birth for reasons such as ovarian aging or altered endometrium receptivity. Literature regarding characteristics of these patients in whom CC medication is ineffective is scant. This becomes increasingly important at the present time since there is an increase in the number of patients who would wish to have a child in a relative later age. Precious time might be lost with ineffective treatment in these patients, which in turn decreases chances of success for subsequent therapies.

Prediction of ovulation induction outcome applying a nomogram may be a step-forward in individualized treatment protocols. The clinician may – after proper external validation - apply these prediction models for decision making in choosing a treatment protocol which may be more effective and less expensive. This issue is becoming also more and more important in the daily clinical practice. Moreover, the social, financial, and economic consequences of a multiple pregnancy (i.e. the recent report on overall live birth in a quadruplet in the US) after ovulation induction should not be ignored. The tremendous consequences of a multiple pregnancy should kept in mind and the aim should be reaching a singleton pregnancy which ends in a live birth.

Step-down regimens may involve a relatively high initial dose of FSH followed by subsequent decremental steps. Particularly for those patients with a low FSH threshold level, the initial standard FSH dose may be too high. This could induce imminent ovarian hyperstimulation requiring cancellation of stimulation (Fauser *et al.*, 1997). Adjusting the starting dose in women with a low FSH threshold would certainly reduce the incidence of multiple dominant follicle development and related complications during the step-down regimen. This could considerably

improve treatment outcome in both low-dose step-up or step-down regimens and render this treatment modality safer and more efficient. Moreover, prediction of patients who are at high risk to develop multiple follicle growth and/or OHSS may be a distinct step forward in reducing the incidence of complication rates after gonadotropin induction of ovulation applying step-up or step-down dose regimens. Moreover, patients who have a poor chance to conceive after gonadotropin ovulation induction may be advised to omit CC therapy and directly start with an alternative treatment modality (such as exogenous gonadotropins, insulin sensitizing hormones, weight reduction, or in vitro fertilization) particularly in women with an advanced age.

From a clinical point of view, it does not seem to matter whether a patient is classified as PCOS or WHO class 2, since all normogonadotropic anovulatory infertile patients start with first line therapy consisting of CC treatment for ovulation induction. Therefore it may be more appropriate to exhibit a shift in attention and focus of various investigations from diagnosis to prognosis as long as no consensus has been reached regarding the definition and diagnosis of PCOS. Applying a distinct end-point (prognosis) such as live birth may be more reliable for the clinician and for the patient. This may render the treatment protocols more cost-effective in a century which financial issue of the hospital and the government has and is going to be more discussed. This "evidence based" approach might lead also to a new sub-classification of the notoriously heterogeneous group of WHO class 2 anovulatory women, which might add to our future understanding of this complex clinical picture.

The prediction of outcome in ovulation induction strategies in WHO 2 patients will be the scope of the present thesis. However, ongoing investigation should elucidate the probability of prediction of outcome of WHO 2 patients who do not reach live birth after CC or gonadotropin induction of ovulation applying a decremental dose regimen.

Chapter II

Prediction of clomiphene citrate ovulation induction outcome

2.1 Introduction

In a longitudinal follow-up cohort study we focused on the predictive value of initial clinical, endocrine, and sonographic screening for ovarian response (ovulation), success (conception as well as live birth), and complications (such as multiple pregnancy or OHSS) following ovulation induction therapies. Various multivariate prediction models have been developed. In this chapter, these models for the prediction of ovarian response and clinical outcome following CC are discussed. This approach may provide valuable information regarding clinical and endocrine factors involved in ovarian dysfunction. Multivariate analysis allows to assess the inter dependence of these factors. Moreover, patients with low chances for success of ovulation induction may be identified in advance.

The first part of the present prospective longitudinal follow-up study was designed to identify whether certain criteria assessed during standardized initial screening could predict the response to ovulation induction with clomiphene citrate (CC) in 201 patients presenting with oligomenorrhea or amenorrhea and infertility. Serum FSH levels were within the normal range (1-10 IU/L), and all patients underwent a spontaneous or progestin-induced withdrawal bleeding. Initial CC doses were 50 mg daily for 5 days starting on cycle day 3. In the case of an absent response, doses were increased to 100 mg and 150 mg daily in subsequent cycles. First ovulation with CC was used as the end point. After a complete follow-up (in the case of a nonresponse, at least 3 treatment cycles with daily CC doses up to 150 mg), 156 patients (78%) ovulated. The free androgen index (FAI = testosterone/sex hormone-binding globulin ratio), body mass index (BMI), cycle history (oligomenorrhea vs. amenorrhea), serum androgen (testosterone and/or androstenedione) levels, and mean ovarian volume assessed by transvaginal sonography were all significantly different ($P < 0.01$) in responders from those in non-responders. FAI was chosen to be the best predictor in univariate analysis. The area under the receiver operating characteristics curve in a multivariate prediction model including FAI, BMI, cycle history, and mean ovarian volume was 0.82. Patients whose ovaries are less likely to respond to stimulation by FSH due to CC treatment can be predicted on the basis of initial screening characteristics, such as FAI, BMI, cycle history (oligomenorrhea or amenorrhea), and mean ovarian volume. These observations may add to ongoing discussion regarding etiological factors involved in ovarian dysfunction in these patients and classification of normogonadotropic anovulatory infertile women.

The second part of the present prospective follow-up study was designed to identify whether clinical, endocrine, or ultrasound characteristics assessed by standardized initial screening of normogonadotropic oligo/amenorrheic infertile

patients could predict conception in 160 women who reached ovulation after clomiphene citrate (CC) medication. Additional inclusion criteria were total motile sperm count of the partner above 1 million and a negative history for any tubal disease. Daily CC doses of 50 mg (increasing up to 150 mg in case of absent ovarian response) from cycle day 3–7 were used. First conception (defined as a positive urinary pregnancy test) was the end point for this study. A cumulative conception rate of 73% was reached within 9 CC-induced ovulatory cycles. Patients who did conceive presented more frequently with lower age ($P < 0.0001$) and amenorrhea ($P < 0.05$) upon initial screening. In a univariate analysis, patients with elevated initial serum LH concentrations (>7.0 IU/L) had a higher probability of conceiving ($P < 0.01$). In a multivariate analysis, age and cycle history (oligomenorrhea vs. amenorrhea) were identified as the only significant parameters for prediction of conception. These observations suggest that there is more to be gained from CC ovulation induction by younger women presenting with profound oligomenorrhea or amenorrhea. Screening characteristics involved in the prediction of ovulation after CC medication in normogonadotropic oligo/amenorrheic patients (body weight and hyperandrogenemia, as shown previously) are distinctly different from predictors of conception in ovulatory CC patients (age and the severity of cycle abnormality). This disparity suggests that the FSH threshold (magnitude of FSH required for stimulation of ongoing follicle growth and ovulation) and oocyte quality (chances for conception in ovulatory cycles) may be differentially regulated.

The third part of the present prospective longitudinal follow-up study was designed to identify whether additional endocrine screening characteristics, all potentially involved in ovarian dysfunction in 182 normogonadotropic oligoamenorrheic infertile women, are associated with ovarian response, which may improve overall prediction of CC-resistant anovulation. Standardized endocrine screening took place before initiation of CC medication (50 mg/day; increasing doses up to 150 mg/day if required) from cycle days 3–7. Screening included serum assays for fasting insulin and glucose, insulin-like growth factor I (IGF-I), IGF-binding protein-1 (IGFBP-1), IGFBP-3, free IGF-I, inhibin B, leptin, and vascular endothelial growth factor. Forty-two women (22% of the total group) did not ovulate at the end of follow-up (a total number of 325 cycles were analyzed). Fasting serum insulin, insulin/glucose ratio, IGFBP-1, and leptin were all significantly different in univariate analyses ($P \leq 0.02$), comparing CC responders *vs.* nonresponders. Forward stepwise multivariate analyses in combination with factors reported earlier for prediction of patients remaining anovulatory after CC revealed a prediction model including 1) free androgen index (FAI = testosterone/sex hormone-binding globulin ratio), 2) cycle history (oligomenorrhea or amenorrhea), 3) leptin level, and 4) mean ovarian volume. These data suggest that decreased insulin

sensitivity, hyperandrogenemia, and obesity, all associated with polycystic ovary syndrome, are prominent factors involved in ovarian dysfunction, preventing these ovaries from responding to stimulation by raised endogenous FSH levels due to CC medication. By using leptin instead of body mass index or waist to hip ratio, the previous model for prediction of patients remaining anovulatory after CC medication could be slightly improved (area under the curve from 0.82–0.85). This may indicate that leptin is more directly involved in ovarian dysfunction in these patients. The capability of insulin and IGFBP-1 to predict patients who remain anovulatory after CC disappears when FAI enters into the model due to a significant correlation between FAI and these endocrine parameters. This suggests that markers for insulin sensitivity (*e.g.* IGFBP-1 and insulin) are associated with abnormal ovarian function through its correlation with androgens, whereas leptin is directly involved in ovarian dysfunction.

2.2 Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligo-amenorrheic infertility

2.2.1 Introduction

Chronic anovulation is a frequent cause of infertility, and approximately 80% of these patients present with serum follicle-stimulating hormone (FSH) and estradiol (E_2) levels within the normal range (WHO group 2) (World Health Organization. *et al.*, 1993). The anti-estrogen clomiphene citrate (CC) is considered to be a successful treatment strategy in these patients. It has been documented that approximately 70-80 % of these women will become ovulatory (MacGregor *et al.*, 1968; Gorlitsky *et al.*, 1978; Shepard *et al.*, 1979; Hammond *et al.*, 1983; Polson *et al.*, 1989; Opsahl *et al.*, 1996), whereas 40-50% of ovulatory women will conceive (Gorlitsky *et al.*, 1978; Hammond *et al.*, 1983).

Polycystic ovary syndrome (PCOS), usually referred to as chronic hyperandrogenic anovulation, represents a distinct proportion of WHO group 2 anovulatory patients. It is uncertain to what extent PCOS patients are particularly prone to remain resistant to CC medication (Lobo *et al.*, 1982b; Adashi *et al.*, 1996). Discussion continues regarding the validity of criteria used to diagnose PCOS (Franks *et al.*, 1995) as well as its relevance for clinical practice. We have previously demonstrated that a distinct overlap exists between endocrine and ultrasound features used by various authors (van Santbrink *et al.*, 1997b). If strict criteria are used for PCOS diagnosis, a large heterogeneous group of normoestrogenic patients will remain unclassified.

Thirty-five years after its first clinical introduction (Greenblatt *et al.*, 1961), CC still remains the first line treatment strategy in normogonadotropic anovulatory patients. Although rising serum FSH levels due to CC interference with estrogen negative feedback may be held responsible for stimulating follicle growth (Jacobson *et al.*, 1968; Miyake *et al.*, 1983; Kerin *et al.*, 1985), other mechanisms of action have also been proposed (Butzow *et al.*, 1995; Adashi *et al.*, 1996). A significant proportion of treated women, however, do not respond. The aim of this study was to investigate whether clinical, endocrine and sonographic characteristics during initial screening of normogonadotropic anovulatory infertile women may predict ovarian response to CC medication. This approach may help to define conditions that prevent the ovary from responding to stimulation by increased FSH levels and to further classify WHO group 2 anovulatory patients.

2.2.2 Subjects and Methods

Subjects and study design

Approval for this study was obtained from the human subject committee of the Dijkzigt Hospital/Erasmus University. Between February 1993 and September 1996, 201 patients presenting with oligomenorrhea (interval between vaginal bleeding >35 days and < 6 months) or amenorrhea (bleeding interval >6 months) and infertility were recruited. Informed consent was obtained from all participants. All subjects were referred directly by their general practitioner to our infertility unit. None had received previous ovulation induction medication. Additional inclusion criteria were serum FSH levels within normal limits (1-10 IU/L) (van Santbrink *et al.*, 1995; World Health Organization. *et al.*, 1993), spontaneous menses or a positive bleeding response to progestagen withdrawal, normal serum PRL and TSH levels, body mass index (BMI; weight divided by the square of the patients height) more than 18, and age less than 40 years.

Clinical, endocrine, and sonographic screening was carried out before initiation of CC therapy. Clinical screening included infertility and cycle history, BMI, previous medication and/or surgery. Endocrine screening included serum assays of FSH, PRL, TSH, LH, estradiol, androstenedione (AD), testosterone (T), sex hormone-binding globulin (SHBG), cortisol, and dehydroepiandrosterone sulfate. Fasting blood samples were taken randomly between 8:00–10:00 A.M. before to initiation of therapy. Venous blood samples were centrifuged within 2 hours after withdrawal and stored at –20 C until assayed. Transvaginal sonographic screening included assessment of the ovarian stroma echogenicity (arbitrarily classified from 1–3), ovarian volume (milliliters), and total number of follicles (both ovaries), as described previously (van Santbrink *et al.*, 1997b; Pache *et al.*, 1992). Serum LH

and FSH levels were measured by immunoradiometric assay (Medgenix, Fleurus, Belgium), and T, AD, SHBG, and dehydroepiandrosterone sulfate were determined using radioimmunoassay (RIA) kits (Diagnostic Products Corp., Los Angeles, CA), as described previously (Fauser *et al.*, 1991; van Dessel *et al.*, 1996).

The treatment schedule and assessment of ovarian response was as follows. CC medication was initiated on day 3 after spontaneous or progestagen-induced withdrawal bleeding. The starting dose was 50 mg/day, orally, for 5 subsequent days. In the case of an absent response, daily doses were increased by 50 mg in the next cycle to a maximum dose of 150 mg/day in the following cycle. If ovulation occurred, the dose remained unaltered during subsequent cycles. First ovulation was used as the end point. The duration of follow-up for all patients included in the study was at least three treatment cycles. Ovulation was assessed by midluteal serum progesterone (P) measurement (levels >25 nmol/L indicating ovulation) combined with transvaginal sonographic monitoring of follicle growth until the appearance of a preovulatory follicle (mean diameter, ≥18 mm) and subsequent follicle rupture, or by biphasic basal body temperature charts. Responders were defined as patients who ovulated during CC therapy, independent of the dose administered. The number of treatment cycles and the CC dose in which first ovulation occurred were recorded. Clomiphene-resistant anovulation (CRA) was defined as patients who do not ovulate despite receiving maximum treatment doses of 150 mg/day.

Data analysis

Distribution of characteristics in patient groups is presented as the mean ± standard deviation (SD). We used the Mann-Whitney U test and the Wilcoxon rank sum W test for exploratory comparison of initial parameters between responders and nonresponders. The univariate and multivariate relation with response to CC was assessed using logistic regression analysis. The following parameters were used in the analysis: BMI, free androgen index (FAI; T x 100/SHBG), serum T and/or AD concentrations, serum LH levels, cycle history (oligomenorrhea or amenorrhea), cycle duration in case of oligomenorrhea, mean ovarian volume, and follicle number. Backward stepwise elimination was used for the multivariate logistic analysis of prediction of patients being CRA, and $P \leq 0.10$ was used as a cut-off level for elimination of nonsignificant predictors from the prognostic model. The area under the receiver operating characteristics (ROC) curve (AUC) was used to assess the discriminative ability of the logistic models. The AUC gives the proportion of all pairs of patients (each pair consisting of one patient without and the other patient with a response to CC) in which the model predicts a higher probability of no response for the patient without response. The Statistical Analysis System program (SAS Institute, Cary, NC) was employed for data analysis.

Because selection and estimation was performed for 8 potential predictors on a dataset with only 45 events (CRAs), correction for overfitting was performed (Harrell, Jr. *et al.*, 1996). The internal validity of the prognostic model was tested by a bootstrapping method in which the selection and estimation process was repeated 200 times. Each of these repetitions consisted of creating a new dataset (bootstrap sample) by drawing cases with replacement from the original data. The backward stepwise elimination process was performed on this dataset, yielding a set of selected predictors and parameter estimates (Van Houwelingen *et al.*, 1990; Harrell, Jr. *et al.*, 1996). Resulting model estimates of each bootstrap sample were evaluated on the original data, and a shrinkage factor was estimated to correct for statistical overoptimism. The Hosmer-Lemeshow goodness of fit test (Hosmer *et al.*, 1989) has been used to check for lack of fit of the final model. The logistic coefficients that were corrected by the shrinkage factor have been translated into an easy to use score chart. The scores were calculated by multiplying the shrunk coefficients by 10 and rounding them off to the nearest whole number.

2.2.3 Results

The number of patients who did or did not ovulate after CC medication in increasing doses of 50, 100, and 150 mg daily are depicted in Figure 1. Forty-five patients (22.5% of the overall study group) remaining anovulatory were considered having CRA. A total of 432 cycles were analyzed.

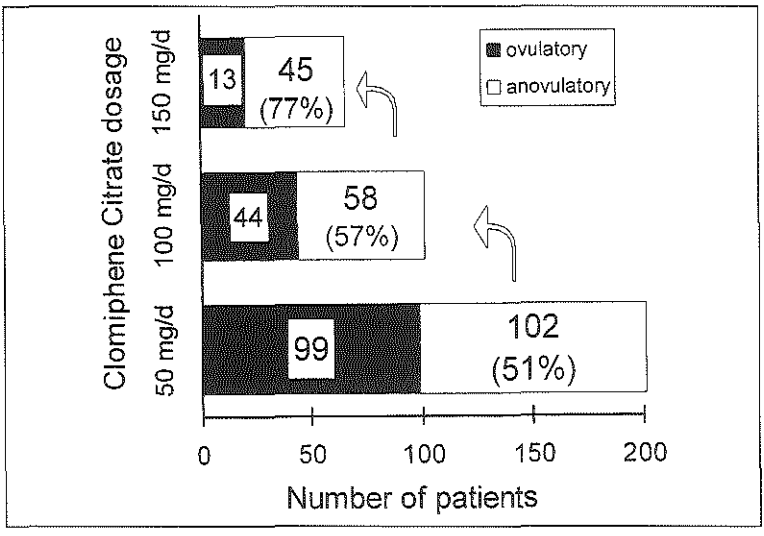

Figure 1 *Distribution of normogonadotropic oligomenorrheic or amenorrheic infertile women who do or do not ovulate following CC induction of ovulation in incremental daily doses of 50, 100, or 150 mg, for 5 subsequent days. A total number of 45 women (22.5% of the overall study group) remain anovulatory.*

From the total study group of 201 women, 91 (46%) were considered obese (BMI >26), 101 patients (50%) presented with an elevated FAI (>4.5), 85 patients (42%) presented with hyperandrogenemia (T ≥3.2 nmol/L and/or AD ≥16.3 nmol/L) (Fauser *et al.*, 1991; van Santbrink *et al.*, 1997b), and in 125 patients (66%) polycystic ovaries (mean ovarian volume ≥10.8 mL and/or mean follicle number per ovary ≥10) (Pache *et al.*, 1992; van Santbrink *et al.*, 1997b) were diagnosed. Finally, 105 patients (54%) presented with elevated LH (≥7.0 IU/L) serum levels (Fauser *et al.*, 1991; van Santbrink *et al.*, 1997b).

In Table 1, clinical, endocrine, and ultrasound characteristics are presented for the overall study group and separately for patients who did or did not ovulate after CC medication. Forty-four percent of patients presenting with amenorrhea (17 of 39) were considered to have CRA, whereas only 17% (28 of 162) of patients with oligomenorrhea showed no response. Statistical significance in univariate analysis with logistic regression analyses and ROC AUC of the initial parameters are depicted in Table 2. The AUCs for FAI and BMI were the highest (0.76 and 0.70, respectively). The ROC curve with the best performance (FAI) and that with the poorest performance (serum LH) are shown in Figure 2.

Of the 201 patients, 187 had complete data on the variables used in the multivariate analysis. Using the backward elimination procedure, 4 variables were selected in the final model: 1) FAI, 2) BMI, 3) cycle history (oligomenorrhea or amenorrhea), and 4) mean ovarian volume. By using the combined information of these 4 variables, the AUC further improved to 0.82 (Table 2 and Figure 2). The bootstrap procedure revealed that these 4 predictors were selected in over two thirds of the bootstrap samples, whereas all other candidate variables were selected in less than half of the samples, which illustrates the stability of the final model. The shrinkage factor was estimated from the bootstrap procedure to be 0.82, indicating that when this study is replicated many times, the resulting coefficients of the final multivariate model are, on the average, 18% smaller. This was incorporated in the calculation of the scores. The scores for different parameters are depicted in Table 3, and resulting probability scores for patients remaining anovulatory after CC medication are shown in Figure 3. The Hosmer-Lemeshow goodness of fit test showed no lack of fit of the final model to the data ($P = 0.3$).

TABLE 1. Clinical, endocrine, and ultrasound characteristics (mean ± SD) during initial screening of 201 normogonadotropic oligomenorrheic or amenorrheic infertile women, and separated for patients who do (responders) or do not ovulate (CRA) after CC induction of ovulation

Screening parameters	Overall group (n = 201)	CC responder (n = 156; 77.5%)	CRA (n = 45; 22.5%)	*P* value[a]
Clinical				
Age (yr)	28 ± 4.4	28 ± 4.5	27.5 ± 4.5	NS
Primary infertility (n)	145 (72%)	110 (71%)	35 (78%)	NS
Amenorrhea (n)	39 (19%)	22 (14%)	17 (38%)	0.0004
Bleeding interval (days, in case of oligomenorrhea)	79 ± 62	70 ± 56	113 ± 72	<0.0001
BMI (kg/m^2)	26.6 ± 6.2	25.5 ± 5.8	30.0 ± 6.6	0.0001
Endocrine				
T (nmol/L)	2.3 ± 0.9	2.1 ± 0.9	2.7 ± 1.0	0.001
AD (nmol/L)	16.5 ± 7.8	15.3 ± 6.5	20.5 ± 10.2	0.001
SHBG (nmol/L)	53 ± 31.7	57 ± 32.5	38.3 ± 23.8	<0.0001
FAI (T x 100/SHBG)	5.9 ± 4.3	4.9 ± 3.4	9.3 ± 5.3	<0.0001
LH (IU/L)	7.8 ± 4.3	7.8 ± 4.3	8.2 ± 4.6	NS
FSH (IU/L)	4.4 ± 1.4	4.5 ± 1.4	4.2 ± 1.4	NS
E_2 (pmol/L)	282 ± 233	296 ± 195	234 ± 78	NS
DHEAS (µmol/L)	7.9 ± 3.8	7.9 ± 3.7	7.9 ± 4	NS
TVS				
Total stroma score[b]	3.0 ± 1.0	2.8 ± 1.3	3.3 ± 1.1	0.006
Mean ovarian vol (mL)	10.0 ± 4.4	9.2 ± 5.7	12.2 ± 5.8	0.0007
Mean follicle no.	11.5 ± 5	11 ± 6	12 ± 5	NS

[a] Comparison of CC responders *vs.* CRA (by Mann-Whitney U test).

[b] Arbitrarily defined as one to three per ovary (both ovaries added).

2.2.4 Discussion

It may be helpful for further classification of normogonadotropic anovulatory infertility and for evaluating pathophysiological factors involved in ovarian abnormalities in these patients to study in a longitudinal fashion whether initial screening parameters may predict success, failure or complications of induction of ovulation. The present study was designed to investigate as a first step whether ovarian response after CC medication could be predicted. It is established in the literature that approximately 75% of patients will ovulate, and less than 50% of the

TABLE 2. Univariate and multivariate logistic regression analyses with score test and area under the ROC curve (AUC) of initial clinical, endocrine, and sonographic screening parameters in 201 normogonadotropic oligomenorrheic or amenorrheic infertile women for the prediction of patients remaining anovulatory after CC induction of ovulation

Parameters	*P* value	AUC[a]
Univariate analyses		
FAI (T x 100/SHBG)	<0.0001	0.76
BMI (kg/m^2)	<0.0001	0.70
Mean ovarian vol	0.0001	0.67
Hyperandrogenemia (elevated T and/or AD)	0.0007	0.64
Oligomenorrhea or amenorrhea	0.0005	0.62
Mean follicle no.	0.1	0.58
Bleeding interval in case of oligomenorrhea	0.42	0.53
LH (IU/L)	0.5	0.52
Multivariate analysis		
Prediction model for CRA[b]		0.82

[a] Area under the ROC curve.

[b] Combination of four initial screening parameters: FAI, BMI, cycle history (oligomenorrhea or amenorrhea), and mean ovarian volume.

total population will conceive after CC as first line medication. If patients remaining anovulatory despite CC therapy could be identified beforehand, ineffective and time-consuming CC medication could be prevented. This may be helpful, particularly for women of advanced reproductive age. Further studies, however, are required to investigate whether alternative primary treatment options are cost effective. Moreover, ethiological factors involved in ovarian dysfunction could be identified in this heterogeneous patient group, as subjects whose ovaries will or will not respond to increased FSH stimulation may be differentiated.

For this first analysis of response to CC medication we decided to focus on ovulation rather than conception. Ovulation is biologically relevant and most closely connected to the desired effects of CC medication. Analysis of conception as the end point requires a comprehensive study of other potential confounders, such as tubal factor, sperm factor, and endometrial function. Four initial screening parameters (FAI, BMI, mean ovarian volume and cycle history) could be identified, predicting patients remaining anovulatory after CC medication. A combination of these parameters showed good predictive power, with a ROC AUC of 0.82. Several studies have been published recently regarding the use of a similar multivariate

model for predicting chances for conception in infertile patients with regular cycles (Eimers *et al.*, 1994; Collins *et al.*, 1995; Snick *et al.*, 1997). Various researchers have investigated the predictive value of clinical and endocrine screening parameters for the response to CC. Only a positive correlation between body weight and the dose of CC required to induce ovulation has been established (Lobo *et al.*, 1982b; Shoham *et al.*, 1990). A recent study has indicated that increased BMI is the only initial parameter that is significantly different between responders and non-responders (Kousta *et al.*, 1997). To our knowledge this is the first time a multivariate prediction model has been applied in the treatment of anovulatory infertility. We could demonstrate that patients suffering from amenorrhea, obesity, increased ovarian volume, and elevated androgens (a complex of signs, symptoms, ultrasound and endocrine findings frequently referred to as PCOS) are most likely to remain anovulatory after CC induction of ovulation.

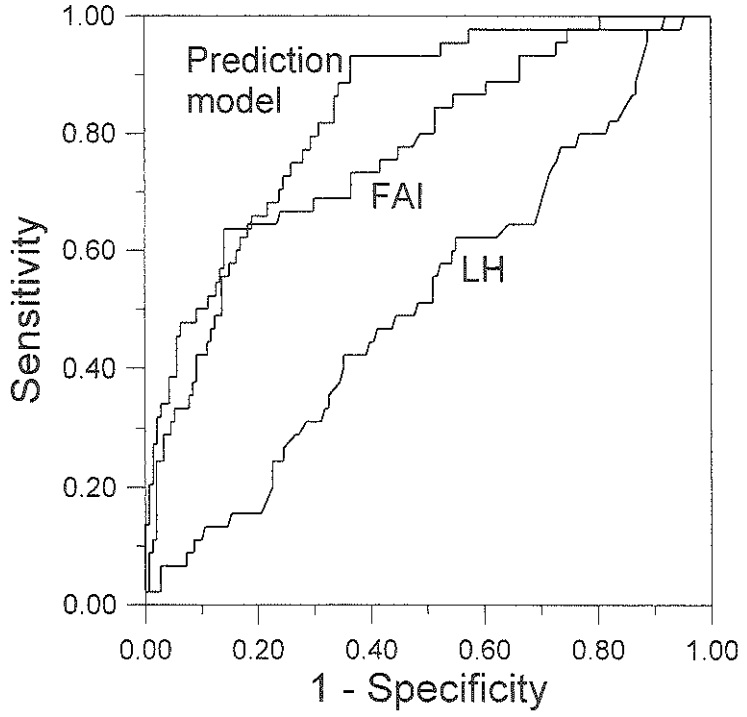

Figure 2 *Receiver Operating Characteristics (ROC) Curve of serum LH concentration, FAI or the prediction model (FAI, BMI, cycle history and mean ovarian volume combined) for predicting clomiphene citrate resistant anovulation (CRA) in a total group of 201 normogonadotropic oligomenorrheic or amenorrheic infertile women.*

A model can be used to predict chances for an individual patient to remain anovulatory by calculating a total score on the basis of these initial screening characteristics. Further studies should validate the prediction model in a new group of patients. The present study also suggests that LH concentrations do not predict ovarian response after CC medication in accordance with recent observations by others (Kousta *et al.*, 1997). These data oppose the concept that elevated LH is implemented in ovarian dysfunction in these patients (Yen *et al.*, 1980). However, the assessment of LH levels in anovulatory patients is problematic due to effects of timing, immunoassays used and the pulsatile nature of LH release (Fauser *et al.*, 1993a).

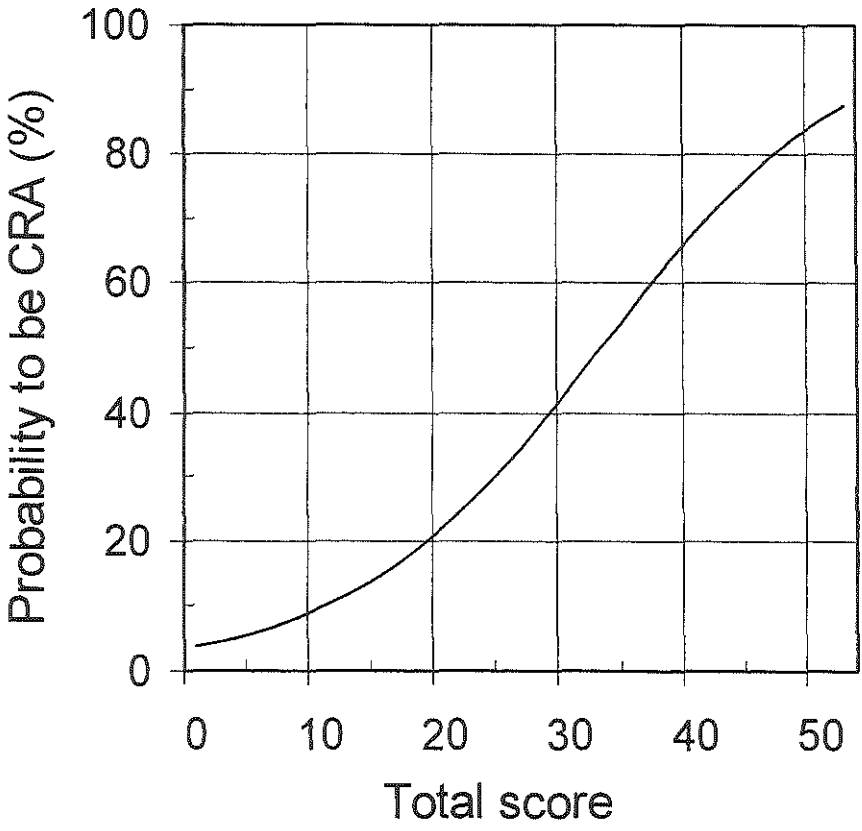

Figure 3 *Score chart and the probability for a given woman to remain anovulatory following CC induction of ovulation.*

TABLE 3. Score charts of four initial screening parameters of the final model for prediction of patients remaining anovulatory after CC induction of ovulation in normogonadotropic oligomenorrheic or amenorrheic infertile women (total scores, 0–53)

Initial screening parameters	Score[a]
FAI (T x 100/SHBG)	
<2	0
2–3	1
3–4	2
4–5	3
5–6	5
6–8	6
8–11	10
>11	14
BMI (kg/m^2)	
<20	0
20–21.5	2
21.5–23	3
23–25	4
25–27	6
27–31	8
31–35	12
>35	15
Mean ovarian vol (mL)	
<6	0
6–7	2
7–8	2
8–9	3
9–11	5
11–13	6
13–16	9
>16	11
Cycle history	
Oligomenorrhea	0
Amenorrhea	13
Total score	-

[a] Encircle the scores related to each category of the screening parameters and add them together. Correspond the total score to the score chart (Fig. 3). As an example, a new amenorrheic patient had the following findings: FAI = 8.7, BMI = 29.4, and mean ovarian volume = 13 ml. Scores are 10 for FAI, 8 for BMI, 9 for mean ovarian volume, and 13 for having amenorrhea. The total score is 40, and the corresponding probability to be CRA is 65%.

Together, these observations suggest that obese hyperandrogenic women are less likely to respond to increased stimulation by FSH, suggesting that these factors are instrumental in follicle maturation arrest (Franks *et al.*, 1998) and an increased FSH threshold (Polson *et al.*, 1989). The correlation between BMI and ovarian response after CC treatment suggests that much emphasis should be focused on weight reduction. However, it should be realized that scientific proof for this approach is lacking, and that weight reduction may not necessarily result in normal response. Previous work from our group demonstrated indeed that long term androgen medication in female to male transsexuals induces polycystic ovary morphology, characterized by increased ovarian size, augmented follicle number, and stroma hyperplasia (Pache *et al.*, 1991). Assessment of steroid levels in follicle fluid obtained from polycystic ovaries suggested that disturbed dominant follicle selection in hyperandrogenic patients may result from disrupted enhancement of FSH-induced aromatase activity (Pache *et al.*, 1992). This could be due to intra-ovarian dysregulation of FSH action, which precludes normal response (*i.e.* follicle growth and ovulation) after incremental FSH levels elicited by CC medication. Factors involved may include locally produced growth factors or insulin resistance (Giudice *et al.*, 1992; Nestler *et al.*, 1997a). Alternative explanations for a nonresponse after CC may include 1) an abnormal hypothalamic / pituitary response to steroid feedback resulting in an insufficient rise in FSH after CC, 2) individual differences in the FSH isohormone profile resulting in discrepancies in bioactive FSH concentrations despite similar immunoreactive FSH levels, 3) additional, as yet unidentified mechanisms responsible for at least part of CC actions. Recently reported CC-induced changes in the insulin-like growth factor system (Butzow *et al.*, 1995) may be relevant in this regard. It may be speculated that improved insight into any of these factors (intraovarian dysregulation (Fauser *et al.*, 1997), hypothalamic / pituitary dysfunction (Berga *et al.*, 1997), FSH heterogeneity (Ulloa-Aguirre *et al.*, 1995), or the insulin-like growth factor system (Giudice *et al.*, 1992)) may eventually result in additional predictors for the CC response. Further studies focusing on insulin resistance may also be of interest, as recent reports suggest that ovarian dysfunction may improve after the use of insulin-sensitizing agents (Sattar *et al.*, 1998). Preliminary observations (Imani, B. and Fauser, B.C.J.M., unpublished observations) suggest that fasting insulin levels are increased in CRA patients. It requires further study to clarify whether insulin resistance is a determining factor in an abnormal response to FSH independent from androgen concentrations, since the majority of studies propose that insulin resistance is associated with PCOS through increased thecal cell androgen production (Nestler *et al.*, 1997a).

In conclusion, this study demonstrates that it is possible to predict patients remaining anovulatory during CC induction of ovulation using criteria that are

directly associated with PCOS, predominantly obesity and hyperandrogenemia. Further studies should establish whether the occurrence of pregnancies after CC medication can also be predicted and whether similar factors are involved. The identification of initial characteristics that predict the ovarian response to ovulation induction therapy may help to further classify the heterogeneous group of normogonadotropic anovulatory infertile women. The present study suggests that hyperandrogenemia and obesity are crucial in inducing ovarian abnormalities that are less likely to respond to increased stimulation by FSH.

2.3 Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility

2.3.1 Introduction

The synthetic antiestrogen clomiphene citrate (CC) represents an easy to use, convenient, inexpensive, and safe first choice medication in normogonadotropic oligo/amenorrheic infertility (WHO group 2) (World Health Organization. *et al.*, 1993). Life-table analysis of pregnancy rates after CC medication and prediction of treatment outcome have been the subject of extensive investigation (MacGregor *et al.*, 1968; Gorlitsky *et al.*, 1978; Shepard *et al.*, 1979; Lobo *et al.*, 1982b; Hammond *et al.*, 1983; Polson *et al.*, 1989; Shoham *et al.*, 1990; Opsahl *et al.*, 1996; Kousta *et al.*, 1997). Cumulative pregnancy rates after CC treatment between 37–97% have been reported (Gorlitsky *et al.*, 1978; Shepard *et al.*, 1979; Hammond *et al.*, 1983). A positive correlation was established between body weight and the CC dose required to induce ovulation (Lobo *et al.*, 1982b; Kousta *et al.*, 1997). Moreover, recent studies have indicated that body mass index (BMI) is significantly higher in nonresponders (Polson *et al.*, 1989; Kousta *et al.*, 1997). Limited information is available, however, concerning the predictive value of initial screening characteristics for treatment outcome (Adashi *et al.*, 1996), and previous investigators were unable to identify predictors for conception after CC induction of ovulation (Hammond *et al.*, 1983; Shoham *et al.*, 1990; Kousta *et al.*, 1997). All the above-mentioned studies focused on the prediction of treatment outcome in the entire group of infertile patients who started with CC medication. In contrast, we focused separately on the prediction of ovulation after CC administration (Imani *et al.*, 1998) and the prediction of conception in ovulatory CC-treated women. This approach seems more appropriate, because statistical bias due to selective drop out from the study for reasons of persistent anovulation despite increasing doses of CC medication is eliminated.

Our group could recently establish that obese hyperandrogenic amenorrheic patients are more likely to be resistant to CC medication (Imani *et al.*, 1998). We now report on initial clinical, endocrine, and sonographic screening characteristics of normogonadotropic oligomenorrheic or amenorrheic infertile women achieving ovulatory cycles after CC medication in an attempt to identify factors predicting chances for conception in these patients. The separate focus on the prediction of conception and ovulation after CC treatment may allow to differentiate among factors affecting oocyte quality independently from follicle development.

2.3.2 Subjects and Methods

Subjects and study protocol

Between February 1993 and December 1997, 160 couples presenting with oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval >6 months) and infertility attending our unit were included in the present study. Additional inclusion criteria were 1) serum FSH levels within normal limits (1–10 IU/L) (World Health Organization. *et al.*, 1993; van Santbrink *et al.*, 1995; Schipper *et al.*, 1998) and normal serum PRL and TSH levels, 2) spontaneous menses or positive bleeding response to progestagen withdrawal, 3) ovulatory cycles after CC induction of ovulation, 4) BMI (weight divided by height squared) greater than 18, 5) age between 19–40 years, 6) a total motile sperm count [TMC = ejaculate volume (milliliters) x sperm concentration (10^6 / mL) x percentage of progressive motile sperm] of the partner above 1 million (Ombelet *et al.*, 1997), 7) negative history for any tubal pathology, and 8) no indication for intrauterine insemination. Study approval was obtained from the human subject committee of the Dijkzigt Hospital/Erasmus University, and informed consent was obtained from all subjects included. A standardized clinical, endocrine, and sonographic screening took place before initiation of induction of ovulation with CC medication.

Ovulation after CC treatment was assessed by midluteal serum progesterone (P) levels above 25 nmol/L, combined with transvaginal sonographic monitoring of follicle growth until visualization of a preovulatory follicle (mean diameter, >18 mm) and subsequent disappearance or biphasic basal body temperature charts, as described previously (Imani *et al.*, 1998). Clomiphene citrate was administered at a daily dose of 50 mg (increased to 100 and 150 mg in subsequent cycles in the case of absent ovarian response) from cycle days 3–7 after initiation of spontaneous or progestin-induced withdrawal bleeding. Conception was defined as a positive urinary pregnancy test (Clearview, hCG II, Unipath Ltd., Bedford, UK) more than 3 days after the expected menses, and ongoing pregnancy was defined as sonographic assessment of an intrauterine gestational sac with positive heart beat.

History and clinical screening included assessment of duration of infertility, whether infertility was primary or secondary, cycle history, previous medication and/or surgery, and BMI. Endocrine screening included serum assays of FSH, LH, estradiol (E_2), testosterone (T), androstenedione (AD), sex hormone-binding globulin (SHBG), and P. Fasting venous blood samples were taken on a random day between 8:00–10:00 A.M., as indicated previously (Imani *et al.*, 1998). Blood samples were centrifuged within 2 hours after withdrawal and were stored at –20 C until assayed. Serum LH and FSH levels were measured by immunofluorometric assay (Amerlite, Ortho-Clinical Diagnostics, Amersham, Aylesbury, U.K.), as described previously (Schipper *et al.*, 1998). P levels were measured by radioimmunoassay (RIA), as described previously (de Jong *et al.*, 1974). Serum E_2, T, AD, and SHBG levels were estimated using RIA kits provided by Diagnostic Products Corp. (DPC, Los Angeles, CA), as described previously (Fauser *et al.*, 1991). Intra- and interassay coefficients of variation were less than 3% and 8% for FSH, less than 5% and 15% for LH, less than 16% and 17% for P, less than 5% and 7% for E_2, less than 3% and 5% for T, less than 8% and 11% for AD, and less than 4% and 5% for SHBG, respectively. Transvaginal pelvic ultrasound (Model EUB-415, Hitachi Medical Corp., Tokyo, Japan) was performed by a single observer (B.I.) and included the assessment of ovarian stroma amount and echogenicity (arbitrarily classified from one to three per ovary), ovarian volume (milliliters), and total number of follicles (both ovaries), as described previously (van Santbrink *et al.*, 1995; Pache *et al.*, 1992). Semen analyses were performed according to WHO guidelines (1992) and comprised ejaculate volume (milliliters), number of spermatozoa per mL (10^6 spermatozoa/mL), percentage of progressive motile spermatozoa, and percentage of normal forms (World Health Organization. *et al.*, 1992).

Data analysis

A *P* value of 0.05 was chosen as the threshold level for statistical significance. Cox regression has been used for life-table analysis of conception rates during CC treatment (Cox *et al.*, 1972). The number of ovulatory CC treatment cycles was the time variable for multivariate analyses. Censoring was defined as definitive discontinuation of CC therapy without conception or end of follow-up (February 1998). To analyze the effect of the severity of the cycle abnormality on chances to conceive after CC treatment, we arbitrarily divided the cycle histories of the patients into four categories; interval between periods of 5–6 weeks (n = 56), 6–9 weeks (n = 50), 9–26 weeks (n = 25), and greater than 26 weeks (*i.e.* amenorrhea) (n = 29). The univariate relation was assessed between the variables listed in Table 1 and the time interval between the first ovulation after CC medication and conception using the Kaplan-Meier method (Kaplan *et al.*, 1958) for categorical and

the Cox regression (Cox *et al.*, 1972) for continuous variables. The Log-rank test has been used to denote statistical significance in life-table analyses. The multivariate analysis was performed with the method of forward stepwise selection to gain a better insight into the interdependence between initial screening parameters. This method can explain why a variable that was significantly different in univariate analysis was not selected in the final model. The prognostic impact of variables was expressed as a fecundability ratio, which is equivalent to the hazard ratio in survival analysis. For instance, a fecundability ratio of 0.9 for an unfavorable group means that the conception rate per ovulatory CC treatment cycle is 10% lower compared to that in the favorable group. In some couples (n = 25), no sperm analysis was performed because of the short time interval between initiation of CC medication and conception. A statistical imputation technique has been applied using multiple conditional mean imputation to fill in these missing sperm parameters (Rubin *et al.*, 1978). The value of semen parameters was estimated using the time until conception and patient characteristics related to sperm parameters in the nonmissing group. Data were analyzed using the commercially available software package SPSS, Inc. (Chicago, IL).

2.3.3 Results

A total of 82 women (51% of the ovulatory group) conceived, and 73 (46%) reached an ongoing pregnancy from the total of 160 patients fulfilling the in/exclusion criteria. Sixty-nine were singleton and 4 were twin pregnancies (data not shown). Initial screening parameters of 9 patients who had a miscarriage after CC (11% of overall CC conceptions) were not different from those of the remaining 73 patients who reached an ongoing pregnancy (data not shown). One woman who conceived (twin pregnancy) during her first CC treatment cycle was frequently monitored on an out-patient basis due to abdominal discomfort and enlarged ovaries. There was no case of severe ovarian hyperstimulation syndrome.

The life-table analysis of cumulative conception rates (CCR) of patients who ovulated after CC are indicated in Figure 1 for the total group, and separately for different dose groups. A cumulative conception rate of 47% was reached within three cycles from first ovulation, and a CCR of 73% was reached within nine CC-induced ovulatory cycles. Patients using daily doses of 50, 100, and 150 mg CC reached cumulative conception rates of 57%, 66%, and 38% within 5 ovulatory cycles, respectively ($P_{\log rank} = 0.25$; Figure 1). At higher doses, chances for conception and ongoing pregnancy are not statistically significantly reduced, although absolute CCR (n = 18) were low in the 150-mg CC group. The overall mean duration of follow-up was 4 ± 3 months and 3.2 ± 2.6 ovulatory CC cycles.

Initial screening characteristics of the overall group of patients who ovulated after CC and separately for those women who conceived (n = 82) *vs.* those who did not (n = 78) are depicted in Table 1. Age, the severity of cycle abnormality (oligomenorrhea *vs.* amenorrhea), and cycle duration, arbitrarily classified in four categories (see also *Materials & Methods*), were significantly different in univariate analysis. Age (cut-off of 30 yr), cycle history (oligomenorrhea *vs.* amenorrhea), and initial serum LH level (cut-off level of 7.0 IU/L) in univariate analyses for CCR are depicted in Figure 2.

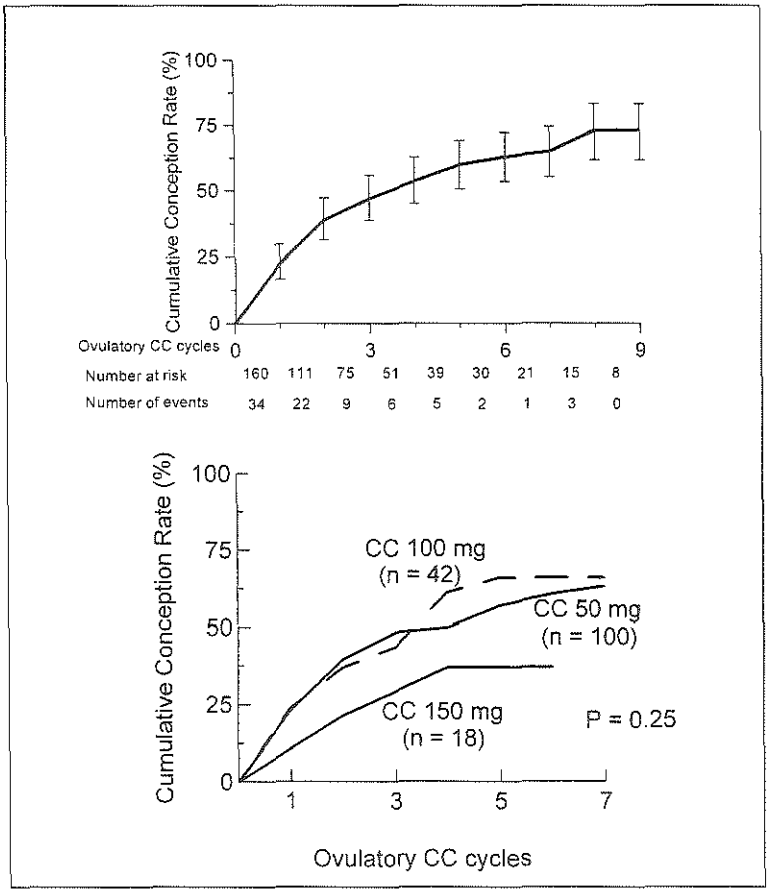

Figure 1 *Life table analysis of cumulative conception rates (CCR) in 160 normogonadotropic oligo-amenorrheic infertile patients who ovulated following CC medication. CCR's (including absolute number of patients at risk and number of events (= conceptions)) are presented for the total study group (upper panel) (vertical lines represents 95% Confidence Intervals), and separately for women who reached ovulatory CC cycles with 50, 100, or 150 mg daily for 5 subsequent days (lower panel). N represents the initial number of patients at risk per dose group. P = Log Rank test P value.*

The percentage of ongoing pregnancies per conception for patients with elevated (initial serum LH level ≥7.0 IU/L) or normal initial serum LH levels were 84.8% and 94.4%, respectively (*P* value for difference in proportion ongoing pregnancies = 0.16, and 95% confidence interval for difference = -3% to 23%). The cut-off value for normal (*i.e.* 7.0 IU/L) was chosen on the basis of a previous study from our group in normoovulatory controls (mean + 1 SD) (van Santbrink *et al.*, 1997b).

A total number of 159 patients had complete data on the variables used in the multivariate analyses. Univariate analysis and forward stepwise multivariate analyses of all initial parameters for the prediction of chances to conceive in ovulatory patients treated with CC are depicted in Table 2. During the stepwise multivariate analysis for the prediction of chances to conceive, age, and cycle history (amenorrhea *vs.* oligomenorrhea) entered into the model (step 1 and 2, respectively). The multivariate-adjusted fecundability ratio for age was 0.90 (95% confidence interval, 0.85–0.95), and that for amenorrhea 0.54 (95% confidence interval, 0.32–0.93).

2.3.4 Discussion

The present prospective follow-up study was designed to evaluate whether initial screening characteristics of 160 normogonadotropic oligo/amenorrheic infertile women could predict conception during ovulatory CC-induced cycles. Although CC medication has been the focus of prevalent research, limited information is available regarding the prediction of conception as treatment outcome (Adashi *et al.*, 1996). Reported cumulative pregnancy rates vary between 37-97%. Most studies, however, suffer from methodological difficulties and different inclusion/exclusion criteria. For the first time, our group has focused on ovulation and conception in separate steps, taking into account that a significant proportion (23%) of patients who remain anovulatory after CC medication (Imani *et al.*, 1998) have no chance of conception. Inclusion of these patients in a study focusing on conception causes statistical bias. This separate focus may offer a better insight into the potential predictive power of initial screening characteristics in a heterogeneous group of normogonadotropic oligo/amenorrheic infertile women (WHO class 2) for CC-induced follicle growth and ovulation, separately from conception.

In the present study, CCR of 63% within six cycles and 73% within nine ovulatory CC-induced cycles have been reached. Two third of patients who conceived reached this end point within the first three ovulatory CC treatment cycles. This is in agreement with previous reports in the literature regarding CC (Garcia *et al.*, 1977; Gorlitsky *et al.*, 1978; Gysler *et al.*, 1982) and is similar to spontaneous conception chances in normoovulatory women (Tietze *et al.*, 1968).

TABLE 1. Initial clinical, endocrine, and ultrasound screening characteristics and sperm parameters of partners (median and range) of 160 normogonadotropic oligomenorrheic or amenorrheic infertile women who ovulated after CC induction of ovulation (overall group) and did or did not (CC failure) conceive

Screening parameters	Overall group (n = 160)	Conceived (n = 82; 51%)	CC failure (n = 78; 49%)	*P* value[a]
Clinical				
Age (yr)	28 ± 4	27 ± 4	29 ± 4	0.0001
Infertility duration (yr)	1.9 ± 2.3	1.6 ± 1.4	2.2 ± 2.9	0.23
Primary infertility (n)	116	62	54	0.40
Amenorrhea (n)	29	18 (62)	11 (38)	0.04
Bleeding interval in 4 categories				0.05[b]
5–6 weeks, n (%)	56 (35)	22	34	
6–9 weeks, n (%)	50 (31)	29	21	
9–26 weeks, n (%)	25 (16)	13	12	
>26 weeks, n (%)	29 (18)	18	11	
BMI (kg/m^2)	26 ± 6	26 ± 6	25 ± 6	0.46
Endocrine				
LH (IU/L)	7.6 ± 4.2	8.2 ± 4.3	6.9 ± 4.0	0.11
T (nmol/L)	2.3 ± 1.0	2.3 ± 1.0	2.3 ± 1.0	0.85
AD (nmol/L)	15.0 ± 6.9	15.8 ± 7.9	14.0 ± 5.7	0.16
FAI[c]	5.4 ± 3.9	5.5 ± 4.1	5.1 ± 3.7	0.20
SHBG (nmol/L)	56 ± 31	54 ± 27	58 ± 35	0.24
E_2 (pmol/L)	259 ± 175	229 ± 145	290 ± 198	0.10
Ultrasound				
Mean ovarian vol (mL)	9.3 ± 3.7	9.2 ± 3.7	9.4 ± 3.7	0.97
Mean follicle number	12 ± 5	12 ± 5	12 ± 5	0.48
Total stroma score[d]	3.2 ± 1.1	3.1 ± 1.1	3.3 ± 1.2	0.66
TMC[e]	73 (1.1–492)	75 (1.5–303)	72 (1.1–492)	0.39[f]
% Normal morphology	24 (1–50)	20 (1–50)	25 (1–50)	0.34[f]

Values are the mean ± SD. Underlined values are statistically significant.

[a] Comparison of CC-conceived *vs.* CC failure (univariate Cox regression).

[b] Chances to conceive differ among four categories of bleeding interval.

[c] FAI = T x 100/SHBG.

[d] Arbitrarily defined as one to three per ovary (both ovaries added).

[e] Total motile sperm count

[f] Computed with multiple imputation of missing values

This is also comparable to conception rates reported for exogenous gonadotropin induction of ovulation (Dor *et al.*, 1980; Hamilton-Fairley *et al.*, 1991; van Santbrink *et al.*, 1995) in anovulatory infertile patients. These observations strongly suggest that the overall detrimental effects of CC on cervical mucus production or endometrial receptivity and subsequent implantation are limited with daily CC doses up to 150 mg. Although the CCR seems to be lower in the high dose CC group, this finding was not statistically significant. It should be noted that the sample size of this group is limited, so actual differences cannot be excluded.

Previous studies were unable to identify predictors for conception in CC induction of ovulation in normogonadotropic infertile women (Hammond *et al.*, 1983; Shoham *et al.*, 1990; Kousta *et al.*, 1997). In the present study, age and cycle history (amenorrhea or oligomenorrhea) were significantly different comparing patients who conceived *vs.* those who did not during CC-induced ovulatory cycles. Multivariate analyses revealed a final model including age and cycle history. The predictive power of age was the highest. The area under the receiver operating characteristics curve of the final model including these two factors reached 0.68 (data not shown), which is substantially lower than that in the previous model predicting ovulation after CC (0.82) (Imani *et al.*, 1998). For reasons of clarity, the forward stepwise approach was chosen (see Table 2). Backward stepwise analysis was also applied, resulting in the same final model (data not shown). Young patients have a higher probability to conceive during CC-induced ovulatory cycles. The fecundability rate of the patient decreases by approximately 10% per year. This is in agreement with reports that indicate that age is an important factor for the prediction of chances for spontaneous conception in untreated normoovulatory subfertile patients (Eimers *et al.*, 1994; Imani *et al.*, 1998; Scott *et al.*, 1995). Similar findings have been reported for the prediction of chances to conceive following exogenous gonadotropin induction of ovulation (Dor *et al.*, 1980) and *in vitro* fertilization treatment (Templeton *et al.*, 1996).

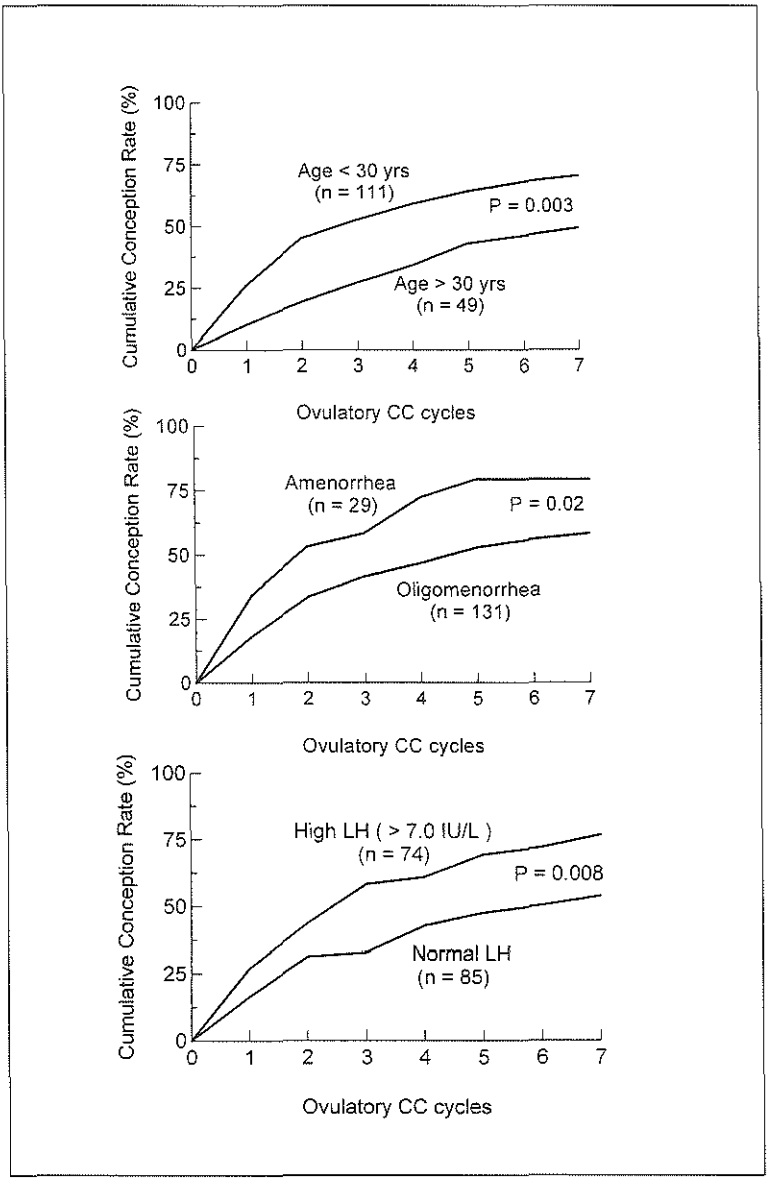

Figure 2 *Univariate analysis of cumulative conception rates in 160 normogonadotropic oligoamenorrheic infertile patients per ovulatory CC cycle. Initial screening parameters shown are: 1) Patient's age (cut-off level at 30 years) (upper panel). 2) Cycle history (oligomenorrhea versus amenorrhea) (middle panel). 3) Initial serum LH concentrations (cut-off level of 7.0 IU/L) (lower panel). n represents the initial number of patients at risk. P = Log Rank test P value.*

TABLE 2. Forward stepwise multivariate analyses of initial screening characteristics for the prediction of chances to conceive in 159 normogonadotropic oligoamenorrheic infertile women who ovulated after CC induction of ovulation

	Univariate:	Multivariate	
Analyses steps	0[a]	1	2
Screening parameters			
Clinical			
Age (yr)	<u>0.0001</u>	In model	In model
Amenorrhea (n = 29)	<u>0.04</u>	<u>0.02</u>	In model
Bleeding interval (in four categories)	<u>0.05</u>	0.06	0.30
Endocrine			
LH (IU/L)	0.11	0.36	0.26
FAI[b]	0.20	0.56	0.38
AD (nmol/L)	0.16	0.38	0.15
E_2 (pmol/L)	0.10	0.26	0.39

Numbers are *P* values for inclusion in the model. *Underlined* numbers are significant at $P < 0.05$.
[a] Only screening parameters with a univariate $P \leq 0.2$ (see Table 1) are shown. In the univariate analysis (step 0), three variables reach statistical significance (*underlined*). In step 1 of the multivariate analysis the variable with the highest prognostic information (age) is selected. After the first step, amenorrhea still reaches statistical significance and, therefore, is selected in the second step. Thereafter, no additional variable is statistically significant anymore, indicating that the model cannot be improved by selecting a subsequent parameter.
[b] FAI = T x 100/SHBG.

Amenorrheic patients exhibit a 2-fold higher probability to conceive after ovulatory CC cycles as compared to oligomenorrheic patients. Patients with longer bleeding intervals also exhibit higher conception chances. We have been unable to find similar reports in the literature regarding induction of ovulation. The most likely explanation seems to be the following. Patients with amenorrhea have an extremely low probability to conceive without intervention due to anovulation before seeking help by a physician. Presumably, the major subfertility factor in these patients is chronic anovulation, which can be resolved temporarily by the use of CC medication. These patients are more likely to be at low risk for any other subfertility factor, such as tubal or sperm dysfunction. Some oligomenorrheic patients may have spontaneous ovulations (Minakami *et al.*, 1988), and some of these women may never seek medical intervention because of spontaneous pregnancies. Their benefit from ovulation induction is an increased chance for conception due to an increased number of ovulations with a fixed interval in a given

period of time. Similar observations were made for pregnancy chances after artificial insemination with donor sperm in relation to the sperm quality of the partner. Donor insemination outcome is significantly better in cases with very poor sperm quality (Emperaire *et al.*, 1982). One should consider that amenorrheic patients also have a higher probability to remain anovulatory after CC, as demonstrated previously (Imani *et al.*, 1998). In contrast, regular ovulatory cycles are easier to induce with CC in oligomenorrheic patients but there is less chance of pregnancy.

In the present study, patients with elevated initial serum LH levels have a significantly higher probability to conceive once ovulatory cycles have been achieved by CC. A recent study also indicated higher initial LH levels in patients who conceived after CC medication (Kousta *et al.*, 1997). In contrast, a poor treatment outcome has been observed in patients with high LH levels during the follicular phase of CC-induced cycles (Shoham *et al.*, 1990). It should be realized that we report on initial LH levels before initiation of CC medication, rather than during CC-induced cycles. Indeed, elevated LH levels may normalize only during CC-induced ovulatory cycles (Eden *et al.*, 1989). These observations are in sharp contrast with reports regarding patients with elevated LH who perform poorly during gonadotropin induction of ovulation (Hamilton-Fairley *et al.*, 1991) or *in vitro* fertilization (Howles *et al.*, 1986). We previously showed that initial LH concentrations did not predict patients who would remain anovulatory after CC medication (Imani *et al.*, 1998). The present observation seems to dispute previous beliefs concerning the detrimental effects of raised LH levels on oocyte maturation and capacity for fertilization. In addition, we did not observe a higher spontaneous abortion rate in patients with high LH levels who conceived after CC medication. This finding is also in contrast with previous reports on the effects of high LH concentrations on chances for spontaneous abortion after exogenous gonadotropins (Homburg *et al.*, 1988; Regan *et al.*, 1990). The predictive power of the initial LH level is poor in case age enters in the final model, which may be due to a correlation of initial LH with age (data not shown). The mechanism of action of CC is not fully elucidated, and the role of LH in the pathogenesis of ovarian abnormalities remains open for speculation. As an example, female siblings of male patients described with an activating mutation of the LH receptor (so-called familial male testotoxicosis) seem to be without a clear phenotype (Fauser *et al.*, 1999).

Total motile sperm count is not a predictor in univariate or multivariate analyses of prediction of conception in the present study population. This is in agreement with the observation that a large overlap exists between semen characteristics of males from fertile *vs.* subfertile couples (Ombelet *et al.*, 1997). Moreover, it can be speculated that couples with better sperm parameters have

already conceived spontaneously before seeking medical intervention.

In summary, it can be concluded that body weight and hyperandrogenemia are the predominant predictors for ovulation after CC treatment, whereas age and cycle history dictate pregnancy chances in ovulatory women. This stresses for the first time the important concept that follicle growth and oocyte quality (and subsequent capacity to be fertilized *in vivo*) are differentially regulated during induction of ovulation, confirming observations during *in vitro* fertilization.

2.4 Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility

2.4.1 Introduction

Clomiphene citrate (CC) is considered to be the first line strategy for ovulation induction in normogonadotropic anovulatory infertility (WHO group 2) (World Health Organization. *et al.*, 1993) since its clinical introduction almost 4 decades ago (Greenblatt *et al.*, 1961). This synthetic steroid, characterized by its mixed estrogenic and antiestrogenic properties, is still extensively used. Approximately 75% of treated patients will ovulate after CC medication (MacGregor *et al.*, 1968; Gorlitsky *et al.*, 1978; Imani *et al.*, 1998), and once ovulation is achieved, cumulative conception rates are 75% within six consecutive CC cycles (Gorlitsky *et al.*, 1978; Shepard *et al.*, 1979; Hammond *et al.*, 1983; Imani *et al.*, 1999).

Polycystic ovary syndrome (PCOS), usually referred to as chronic hyperandrogenic anovulation (Dunaif *et al.*, 1992), is a common endocrine disorder and represents a significant proportion of normogonadotropic anovulatory infertility (van Santbrink *et al.*, 1997b). Etiological factors involved in ovarian dysfunction in this heterogeneous patient group have not yet been fully elucidated. Various investigators reported on the prominent role of locally produced growth factors, such as insulin-like growth factors (IGFs) (Giudice *et al.*, 1996; Cataldo *et al.*, 1997; van Dessel *et al.*, 1999) or vascular endothelial growth factor (VEGF) (Agrawal *et al.*, 1998a). Moreover, recent evidence suggests that decreased insulin sensitivity plays an important role in initiating ovarian dysfunction in these patients, possibly by stimulating thecal cell androgen production (Dunaif *et al.*, 1997; Nestler *et al.*, 1998). A significant proportion of patients with PCOS suffer from severe obesity (Dunaif *et al.*, 1988; Pasquali *et al.*, 1993), and therefore, leptin, the hormone product of the obesity gene (Zhang *et al.*, 1994), has been the focus of intense investigation (Cohen *et al.*, 1996; Brzechffa *et al.*, 1996; Rouru *et al.*, 1997; Laughlin

et al., 1997). It has been demonstrated that leptin can impair insulin action in hepatocytes (Cohen *et al.*, 1996). Although leptin does not appear to play a direct role in either hyperandrogenemia or hypersecretion of LH (Laughlin *et al.*, 1997), abnormalities in its signaling may be involved in hypogonadotropic hypogonadism (Clement *et al.*, 1998) and in certain cases of PCOS (Brzechffa *et al.*, 1996). Finally, inhibin B, which has been shown to be produced by healthy antral follicles under the control of FSH in the early follicular phase of the normal menstrual cycle (Groome *et al.*, 1996), may serve as a marker for the severity of ovarian dysfunction in PCOS patients (Pache *et al.*, 1992; Anderson *et al.*, 1998).

We previously demonstrated that obese, hyperandrogenic, amenorrheic women are less likely to respond to stimulation by increasing endogenous FSH levels after CC compared to normal weight, oligomenorrheic women presenting with normal androgen concentrations (Imani *et al.*, 1998). This approach may have clinical implications in terms of health economics, but may also provide information regarding factors involved in ovarian dysfunction in these women. We now report on the predictive power of additional endocrine screening of factors potentially involved in ovarian abnormalities in this patient population, including insulin sensitivity, the IGF system, leptin, inhibin B, and VEGF.

2.4.2 Materials and Methods

Subjects and study protocol

Between February 1993 and January 1998, 182 women attending our infertility unit presenting with 1) oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval >6 months), 2) serum FSH levels within normal limits (1–10 IU/L) (World Health Organization. *et al.*, 1993; van Santbrink *et al.*, 1995) and normal serum PRL and TSH levels, 3) spontaneous menses or positive bleeding response to progestagen withdrawal, 4) body mass index (BMI; weight divided by height squared) more than 18 kg/m^2, and 5) age between 19–40 years were included in the present study. Study approval was obtained from the human subjects committee of the Dijkzigt Hospital/Erasmus University, and informed consent was obtained from all subjects.

Standardized initial clinical, sonographic, and endocrine screening took place before initiation of induction of ovulation with CC medication, as described previously (Imani *et al.*, 1998). Clinical screening included age, infertility and cycle history, BMI, waist to hip ratio (WHR), previous medication, and/or surgery. Transvaginal sonographic screening included assessment of the ovarian stroma echogenicity (arbitrarily classified from one to three per ovary), ovarian volume (milliliters), and total number of follicles (both ovaries), as described previously

(van Santbrink *et al.*, 1997b; Pache *et al.*, 1992). Endocrine screening included serum assays for FSH, PRL, TSH, LH, estradiol (E_2), androstenedione (AD), testosterone (T), and sex hormone-binding globulin (SHBG), as described previously (Imani *et al.*, 1998). In addition, serum was assayed for fasting insulin and glucose, free and total IGF-I, IGF-binding protein-1 (IGFBP-1), IGFBP-3, inhibin B, leptin, and VEGF concentrations. Fasting venous blood samples were taken on a random day between 8:00–10:00 A.M., before initiation of therapy. Blood samples were centrifuged within 2 hours after withdrawal and stored at –20 C until assayed.

Hormone assays

Serum insulin levels were measured by immunoradiometric assay (IRMA; Biosource Technologies, Inc. Fleurus, Belgium), glucose levels by the hexokinase method (Gluco-quant, Roche Molecular Biochemicals, Mannheim, Germany) (Neeley *et al.*, 1972), free IGF-I levels by a two-site IRMA (Active, Diagnostic Systems Laboratories, Inc., Webster, TX) direct assay. Serum was incubated in tubes and decanted, and the tubes washed according to the manufacturer's instructions. Free IGF-I, bound to the tube, was detected by radiolabeled antibody as described previously (van Dessel *et al.*, 1996). Serum IGF-I levels were measured using a two-site IRMA (Active, Diagnostic Systems Laboratories, Inc.), which includes an extraction step to separate the IGF-I from its binding protein in serum. IGFBP-1 and IGFBP-3 levels were assayed using a two-site IRMA (Active, Diagnostic Systems Laboratories, Inc.) assay as described previously (van Dessel *et al.*, 1996). The IGFBP-3 samples were diluted 1:100 before assay. Dimeric inhibin B levels were measured using an immunoenzymometric assay (Serotec, Oxford, UK) (Schipper *et al.*, 1998), leptin levels using a radioimmunoassay (RIA) (by means of reagents supplied by Linco Research, Inc., St. Charles, MO) (Lahlou *et al.*, 1997), and VEGF levels by enzyme immunoassay (Cytokit Red^{TM} EIA kits; Peninsula Laboratories, Inc., College Park, MD) (Agrawal *et al.*, 1998b). Midluteal serum progesterone (P) levels were measured by RIA, as described previously (Imani *et al.*, 1999). Intra- and interassay coefficients of variation were less than 6.1% and 9.9% for insulin, less than 1.0% and 1.9% for glucose, less than 10.3% and 10.7% for free IGF-I, less than 3.4% and 8.2% for IGF-I, less than 5.2% and 6.0% for IGFBP-1, less than 3.9% and 1.9% for IGFBP-3, less than 8% and 14% for inhibin B, less than 3.6% and 4.6% for leptin, and less than 7.8% and 12.2% for VEGF, respectively.

CC ovulation induction protocol

CC was administered at a daily dose of 50 mg (which was increased to 100 and 150 mg in subsequent cycles in the case of absent ovarian response) from cycle days 3–7 after initiation of a spontaneous or progestin-induced withdrawal bleeding, as

described previously (Imani *et al.*, 1998). Ovulation after CC treatment was assessed by midluteal serum P levels above 25 nmol/L combined with transvaginal sonographic monitoring of follicle growth until visualization of a preovulatory follicle (mean diameter, >18 mm) and subsequent disappearance or biphasic basal body temperature charts, as indicated previously (Imani *et al.*, 1998). If ovulation occurred, the CC dose remained unaltered during subsequent cycles. The duration of follow-up for all patients included in the study was at least three treatment cycles. First ovulation after CC was used as the endpoint. Responders were defined as patients who ovulated after CC, independent of the dose administered. The number of treatment cycles and the CC dose in which first ovulation occurred were recorded. Clomiphene-resistant anovulation (CRA) was defined as patients who did not ovulate despite receiving maximum treatment doses of 150 mg/day.

Data analysis

$P = 0.05$ was chosen as threshold level for statistical significance in univariate analyses. For comparison of initial screening parameters of CC responders and nonresponders, we used the Mann-Whitney U test and Wilcoxon rank sum W test. The univariate relation with response to CC was assessed using logistic regression analysis. The following initial serum parameters were used in the multivariate analyses (forward stepwise selection of variables, $P_{entry} < 0.10$): fasting serum insulin and glucose, IGF-I, IGFBP-1, IGFBP-3, free IGF-I, inhibin B, leptin, and VEGF in combination with parameters of the previous model for prediction of patients remaining anovulatory after CC treatment including FAI, cycle history (oligomenorrhea *vs.* amenorrhea), BMI, and mean ovarian volume (Imani *et al.*, 1998). The area under the receiver operating characteristics curve (AUC) was used to assess the discriminative ability of the tests, as described previously (Imani *et al.*, 1998). Data were analyzed using the commercially available software package SPSS, Inc. (Chicago, IL).

2.4.3 Results

From the total number of 182 patients fulfilling the in/exclusion criteria and treated with CC medication, 131 (72%) suffered from primary infertility, 43 (24%) from amenorrhea and the remaining 139 (76%) had oligomenorrhea. Eighty two (45%) women were obese (BMI, ≥26), 113 (62%) patients presented with an elevated FAI (FAI ≥4.0), and 67 (37%) patients presented with hyperandrogenemia (testosterone, ≥3.2 nmol/L; and/or androstenedione, ≥16.3 nmol/L) (Fauser *et al.*, 1991; van Santbrink *et al.*, 1997b). In 134 patients (74%) polycys-

tic ovaries (mean ovarian volume, ≥10.8 mL; and/or mean follicle number per ovary, ≥10) (Pache *et al.*, 1992; van Santbrink *et al.*, 1997b) were diagnosed.

TABLE 1. Endocrine findings (mean ± SD) upon initial screening of 182 normogonadotropic oligoamenorrheic infertile women[a] and separately for patients who did (CC responders) or did not (CRA) ovulate after CC induction of ovulation

	Overall group (n = 182)	CC responder (n = 142; 78%)	CRA (n = 40; 22%)	P value [b]
Insulin (mU/L)	14.7 ± 10.4	13.6 ± 9.5	19.0 ± 13.0	0.009
Glucose (mmol/L)	4.3 ± 1.6	4.2 ± 1.0	4.8 ± 2.9	— [c]
Insulin/glucose ratio	3.6 ± 2.5	3.4 ± 2.3	4.4 ± 3.0	0.05
Inhibin B (ng/L)	153 ± 102	156 ± 103	149 ± 99	—
IGF-I (ng/mL)	237 ± 97	242 ± 99	218 ± 83	—
Free IGF-I (ng/mL)	2.8 ± 1.7	2.8 ± 1.6	3.0 ± 2.0	—
IGFBP-1 (ng/mL)	24.2 ± 18.1	26.4 ± 18.5	16.7 ± 14.5	0.001
IGFBP-3 (ng/mL)	3297 ± 693	3320 ± 714	3240 ± 622	—
Leptin (ng/mL)	21.6 ± 17	19.3 ± 15	30.4 ± 20	0.003
VEGF (ng/mL)	4.8 ± 2.9	4.9 ± 2.9	4.3 ± 3.1	—

[a] Remaining characteristics: age, 28 ± 4.5 yr; BMI, 26.5 ± 6 kg/m^2; T, 2.4 ± 0.9 nmol/L; FAI, 6.2 ± 4.2; LH, 7.9 ± 4.4 IU/L; FSH, 4.5 ± 4.6 IU/L; E_2, 258 ± 174 pmol/L; and mean ovarian volume, 9.8 ± 4.2 mL.

[b] Comparison of CC responders *vs.* CRA (by Mann-Whitney U test).

[c] Not significant.

Finally, 93 patients (51%) presented with elevated LH levels (≥7.0 IU/L) (Fauser *et al.*, 1991; van Santbrink *et al.*, 1997b). From the total group of patients, 40 (22%) women remained anovulatory despite the maximum CC dose. A total number of 325 CC cycles were analyzed (205 ovulatory and 120 anovulatory cycles).

Endocrine screening characteristics of the overall study group and separately for patients who did or did not ovulate after CC medication are presented in Table 1. Fasting insulin level, insulin/glucose ratio, and serum leptin levels were significantly higher and IGFBP-1 levels significantly lower in CRA patients. *P* values after univariate analysis with logistic regression analyses, odds ratio, 95% confidence interval, and the receiver operating characteristics AUC of additional initial endocrine screening parameters are depicted in Table 2. Again serum insulin, insulin/glucose ratio, IGFBP-1, and leptin levels were significantly different between patients who did *vs.* those who did not ovulate after CC treatment. Results

of multivariate analyses for prediction of patients remaining anovulatory after CC induction of ovulation are depicted in Table 3. Using a forward stepwise selection procedure, various significant predictors during univariate analysis (such as insulin, IGFBP-1, and BMI) disappear, and four variables were eventually selected: 1) FAI, 2) cycle history (oligomenorrhea *vs.*amenorrhea), 3) leptin, and 4) mean ovarian volume. The association of FAI, leptin and insulin with other endocrine and clinical features was analyzed in more detail. Correlations between FAI and BMI, leptin, insulin, and IGFBP-1 are depicted in Figure 1. Correlations between initial serum leptin levels and BMI, WHR, and insulin are shown in Figure 2. Correlations between initial serum insulin levels and BMI, SHBG, and IGFBP-1 are shown in Figure 3. For all figures, individual correlations are shown separately for CC responders and nonresponders. With exception of leptin, correlations between all other factors depicted in the figures were significantly stronger in CC nonresponders than in responders (data not shown).

2.4.4 Discussion

The present prospective follow-up study was designed to evaluate whether initial screening of insulin sensitivity, the IGF system, leptin, inhibin B, or VEGF could predict the ovarian response to CC medication in normogonadotropic oligoamenorrheic infertile women. We recently reported (Imani *et al.*, 1998) that obese, hyperandrogenic, amenorrheic women, a complex of signs and symptoms frequently referred to as PCOS (Dunaif *et al.*, 1992), are more likely to remain anovulatory after CC medication. As a first step we focused on screening of the most pertinent clinical, endocrine, and sonographic characteristics of these patients (Imani *et al.*, 1998). Further endocrine parameters potentially related to ovarian dysfunction in these women are the focus of the present study. Decreased insulin sensitivity / hyperinsulinemia or augmented free IGF-I may be involved in the pathophysiology of this heterogeneous syndrome by directly stimulating thecal cell androgen production (Dunaif *et al.*, 1997; van Dessel *et al.*, 1999; Nestler *et al.*, 1998). Serum leptin levels were also assessed in these patients, as it has been suggested recently that this is a reliable endocrine marker for obesity. Abnormalities in its signaling pathway may be involved in hypogonadotropic hypogonadism (Clement *et al.*, 1998) and eventually in certain cases of PCOS (Brzechffa *et al.*, 1996; Rouru *et al.*, 1997). In addition, leptin may directly modulate insulin activity in obese individuals (Cohen *et al.*, 1996). Inhibin B is produced by healthy early antral follicles under the control of FSH in the early follicular phase of the menstrual cycle (Groome *et al.*, 1996) and may therefore be used as a marker for the severity of ovarian abnormalities (*i.e.* the sensitivity to FSH and assessment of the

number of healthy, rather than atretic, follicles in polycystic ovaries) and the likelihood of ovaries to respond to ovulation induction (Anderson *et al.*, 1998). Finally, VEGF levels were assessed in these patients, as recent reports suggest that this protein may serve as a marker for changes in ovarian stroma of PCOS patients (Agrawal *et al.*, 1998b; Agrawal *et al.*, 1998a).

TABLE 2. Univariate logistic regression analyses, odds ratio, 95% confidence interval, and area under the ROC curve of endocrine screening parameters in 182 normogonadotropic oligoamenorrheic infertile women for the prediction of patients remaining anovulatory after CC induction of ovulation

Univariate analyses	Odds ratio (95% CI[1])	P[2] value	AUC[3]
Insulin (mU/L)	1.05 (1.01–1.08)	**0.005**	0.64
Glucose (mmol/L)	1.21 (0.97–1.50)	0.07	0.51
Insulin/glucose ratio	1.16 (1.01–1.32)	**0.03**	0.61
Inhibin B (ng/L)	0.99 (0.99–1.00)	0.5	0.53
IGF-I (ng/mL)	0.99 (0.99–1.00)	0.2	0.56
Free IGF-I (ng/mL)	1.06 (0.86–1.30)	0.3	0.51
IGFBP-1 (ng/mL)	0.96 (0.93–0.98)	**0.01**	0.67
IGFBP-3 (ng/mL)	0.99 (0.99–1.00)	0.6	0.54
Leptin (ng/mL)	1.03 (1.01–1.06)	**0.0003**	0.70
VEGF (ng/mL)	0.92 (0.79–1.07)	0.4	0.58

[1] Confidence interval.

[2] Univariate logistic regression analyses with likelihood ratio test (**Bold** = $P < 0.05$).

[3] Area under the ROC curve.

The present study demonstrates that serum insulin, insulin/glucose ratio, and leptin levels are significantly higher and, in contrast, IGFBP-1 levels are lower in patients who remain anovulatory after CC. When a multivariate analysis for the prediction of CRA is applied, the capacity of serum insulin, insulin/glucose ratio, and IGFBP-1 levels to predict CRA is eliminated when FAI enters into the model due to a strong correlation between these parameters with FAI (Table 3, and Figure 1). In contrast, leptin levels enter into the model in the third step. These results can be interpreted as follows. Insulin is significantly higher in CRA patients. An association between decreased insulin sensitivity and hyperandrogenism is well known (Poretsky *et al.*, 1991; Poretsky *et al.*, 1996). Signal transduction via the insulin receptor has been demonstrated to be frequently abnormal in PCOS (Legro *et al.*, 1998). Moreover, IGF-I as well as

insulin have been shown to augment LH-induced androgen biosynthesis by cultured thecal cells (Legro *et al.*, 1998). As insulin binds to the structurally similar type 1 IGF receptor, it has been postulated that high levels of circulating insulin stimulate thecal cell androgen production through the type 1 IGF receptors (Poretsky *et al.*, 1991; Poretsky *et al.*, 1996). However, this seems to be the case only in extreme hyperinsulinism, such as in patients suffering from leprechaunism. Moreover, insulin and BMI are the major determining factors of circulating IGFBP-1 levels in both normoovulatory obese and PCOS women. Insulin reduces circulating SHBG (Weaver *et al.*, 1990; Nestler *et al.*, 1991; Yki-Jarvinen *et al.*, 1995) and IGFBP-1 levels by inhibiting the production of these proteins in the liver (Weaver *et al.*, 1990; Yki-Jarvinen *et al.*, 1995). Apparently, the liver is excluded from reduced sensitivity for insulin stimulation. Accordingly, it has been proposed that IGFBP-1 levels may be used as a simple marker for insulin resistance (Tiitinen *et al.*, 1993). Several researchers indicated lower circulating levels of IGFBP-1 (Weaver *et al.*, 1990; Zachow *et al.*, 1997; Agarwal *et al.*, 1999), but not IGFBP-3 (Agarwal *et al.*, 1999), in PCOS patients compared to normal cycling women (Lahlou *et al.*, 1997; Agrawal *et al.*, 1998b; Fauser *et al.*, 1991; Tiitinen *et al.*, 1993). Moreover, insulin may also inhibit IGFBP-1 production in ovarian granulosa cells, by acting through its own receptor (Poretsky *et al.*, 1996). Circulating serum SHBG concentrations are significantly reduced in obese normoovulatory women (Weaver *et al.*, 1990) and in oligoamenorrheic CC-resistant infertile patients (Imani *et al.*, 1998). Moreover, an increase in circulating serum SHBG concentration has been reported in ovulatory patients after CC medication (Butzow *et al.*, 1995) as well as in women with PCOS using insulin-sensitizing agents (Nestler *et al.*, 1997b; Dunaif *et al.*, 1996). An insufficient or absent rise in circulating serum SHBG concentrations (with resulting high free androgen levels) after CC medication could be held responsible for CC unresponsiveness.

In the present study total and free IGF-I and IGFBP-3 levels are comparable between CC responders and nonresponders. No differences in IGFBP-3 levels were previously noted in follicular fluid from PCOS ovaries compared with androgen-dominant and estrogen-dominant follicles from normoovulatory women (Cataldo *et al.*, 1992; San Roman *et al.*, 1992). In contrast, IGFBP-1 levels are significantly lower in patients remaining anovulatory after CC medication. As a consequence of decreased hepatic production of serum IGFBP-1 levels, a greater proportion of IGF-I is biologically active (LeRoith *et al.*, 1995). Taking into account that serum IGFBP-1 levels increase (Tiitinen *et al.*, 1993) and IGF-I levels decrease (Butzow *et al.*, 1995) in oligoamenorrheic women after CC medication, it may be suggested that an insufficient rise in serum IGFBP-1

levels after CC medication is involved in the follicle growth arrest in these patients. No difference could be observed in pretreatment free IGF-I levels comparing responders *vs.* CRA patients. Overall, FAI is the most significant factor to predict ovarian response. It appears that the prediction of insulin and IGFBP-1 completely depends on its association with FAI, in line with recent compelling molecular evidence for intrinsic abnormalities in the androgen synthesis pathway in PCOS thecal cells (Nelson *et al.*, 1999). It is likely that decreased IGFBP-1 is an excellent marker of hyperinsulinemia and that it does not affect the endocrine IGF system's effect on follicle growth.

The present study demonstrates a significant correlation between leptin levels and BMI and WHR, which is in agreement with previous reports (Laughlin *et al.*, 1997; Rouru *et al.*, 1997; Brzechffa *et al.*, 1996). Serum leptin levels were significantly higher in CC nonresponders and enter into the model (instead of BMI) in multivariate analyses to predict CRA (Table 3). The AUC of the previously developed prediction model (including FAI, BMI, cycle history, and mean ovarian volume) of 0.82 could be slightly improved to 0.85 by the inclusion of leptin rather than BMI. Due to the limited improvement of the AUC and the fact that information such as weight and height of the patient is easy to collect, one may prefer to use BMI to predict the probability to be CRA in clinical practice. Clearly, the end point for clinical practice should be pregnancy rather than ovulation. It has been demonstrated that leptin can directly impair insulin action in hepatocytes (Cohen *et al.*, 1996). In addition, leptin directly inhibits IGF-I augmentation of FSH-stimulated estradiol production (Zachow *et al.*, 1997) as well as LH-stimulated androgen synthesis *in vitro* (Zachow *et al.*, 1997). *In vivo* observations, however, would argue against a negative correlation between obesity / elevated leptin levels and androgens. High leptin levels could interfere with the ability of the dominant follicle to produce estradiol, both by inhibiting the production of androgen substrate and by decreasing the aromatizing capacity of granulosa cells (Agarwal *et al.*, 1999). Moreover, changes in estradiol and progesterone during the human menstrual cycle may participate in the control of leptin production (Messinis *et al.*, 1999). Caloric restriction, exercise, and weight-reducing diets may reduce leptin levels (Carantoni *et al.*, 1999); result in normalization of insulin sensitivity, gonadotropins, and androgen metabolism (Kiddy *et al.*, 1989); and lead to spontaneous ovulatory cycles in up to 50% of women (Hollmann *et al.*, 1996).

Initial serum inhibin B as well as serum VEGF levels were similar comparing CC responders and nonresponders. These endocrine parameters had no predictive value for ovarian response to CC medication.

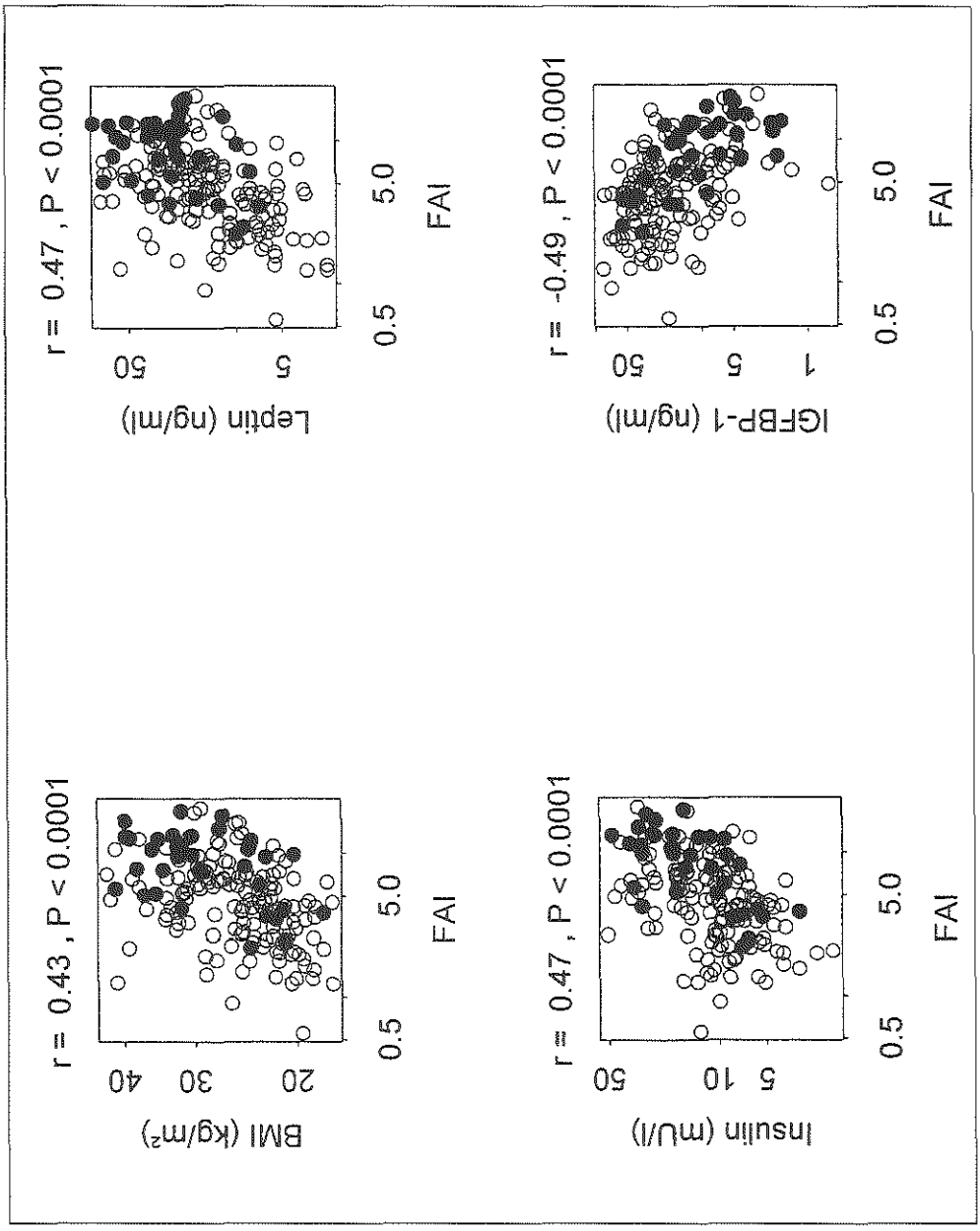

Figure 1 *Correlations between free androgen index (FAI = T x 100/ SHBG) and BMI, serum leptin, insulin, and IGFBP-1 in 182 normogonadotropic oligoamenorrheic infertile patients using scatter plot. Open circles represent patients who ovulated following CC, and closed circles CC-non responders.*

TABLE 3. Forward stepwise multivariate analyses of parameters of the prediction model plus additional endocrine screening for prediction of normogonadotropic oligoamenorrheic patients remaining anovulatory during clomiphene citrate induction of ovulation

Analyses:	Univariate[a]	Multivariate			
Steps:	0	1	2	3	4[b]
Screening parameters					
a) Prediction model[c]					
FAI[d]	<0.0001	In model	In model	In model	In model
BMI (kg/m^2)	0.0003	0.03	0.04	0.46	0.46
Mean ovarian vol (mL)	0.007	0.12	0.13	0.06	In model
Cycle history[e]	0.0003	0.0001	In model	In model	In model
b) Additional endocrine parameters[f]					
Insulin (mU/L)	0.005	0.32	0.45	0.93	0.98
Insulin/glucose ratio	0.03	0.66	0.79	0.64	0.56
IGFBP-1 (ng/mL)	0.01	0.82	0.71	0.74	0.64
Leptin (ng/mL)	0.0003	0.01	0.009	In model	In model

[a] Step 0, Comparison of CC responders *vs.* CRA (univariate logistic regression analyses).

[b] Final model, Area under the ROC curve, 0.85; multivariate odds ratio (95% CI) of FAI, 1.19 (1.07–1.32); of cycle history, 0.16 (0.05–0.42); of mean ovarian volume, 1.11 (0.99–1.24); and of leptin, 1.04 (1.01–1.06). This means that, for instance, a 1-U increase in a given patient's serum leptin level gives her a 4% higher probability to be a CRA.

[c] As published previously (5).

[d] FAI = T x 100/SHBG.

[e] Amenorrhea *vs.* oligomenorrhea.

[f] Only parameters that are significantly different (see Table 2) are included.

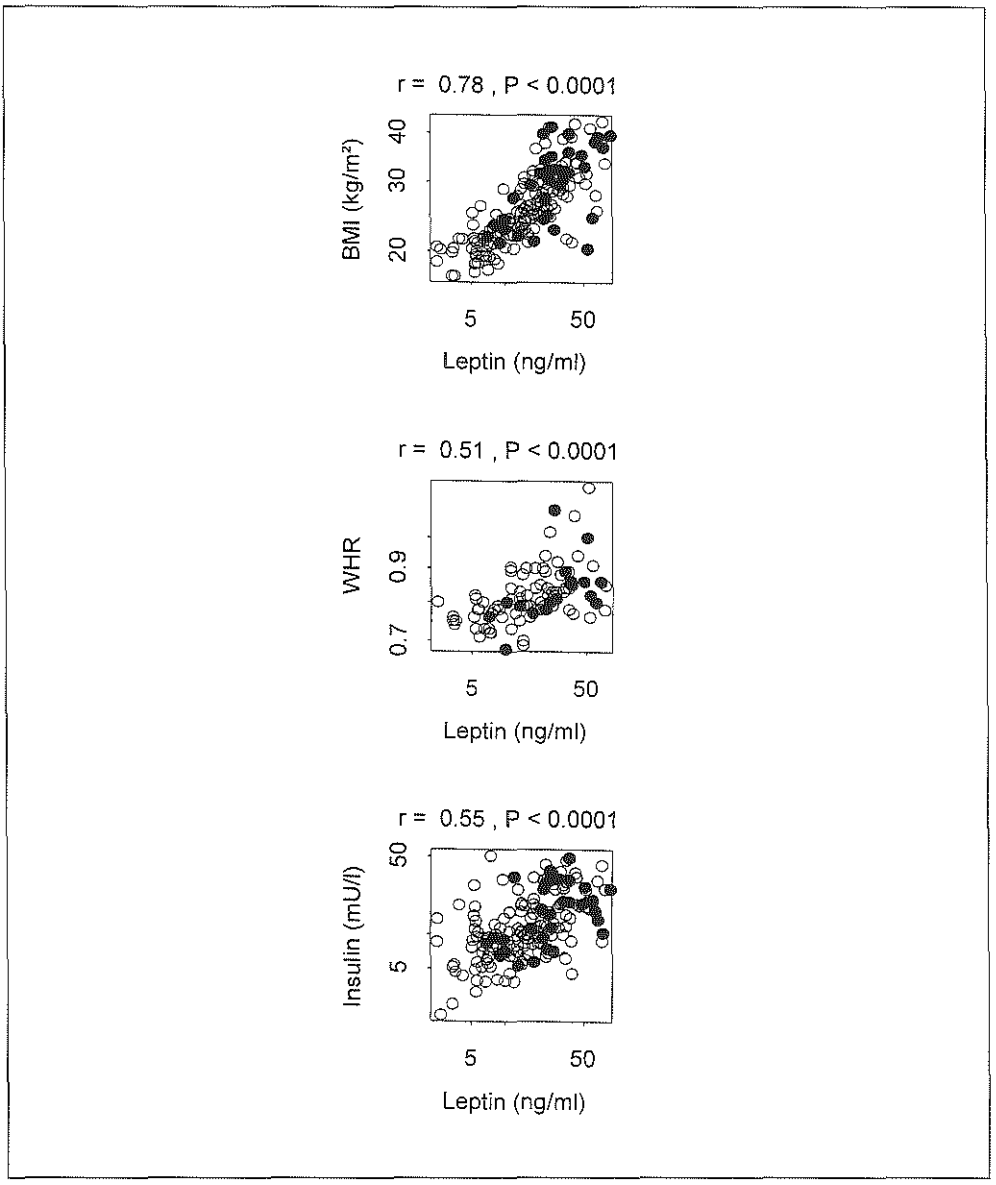

Figure 2 *Correlations between serum leptin and BMI, waist-to-hip ratio (WHR), and serum insulin in 182 normogonadotropic oligoamenorrheic infertile patients using scatter plot. Open circles represent patients who ovulated following CC, and closed circles CC-non responders.*

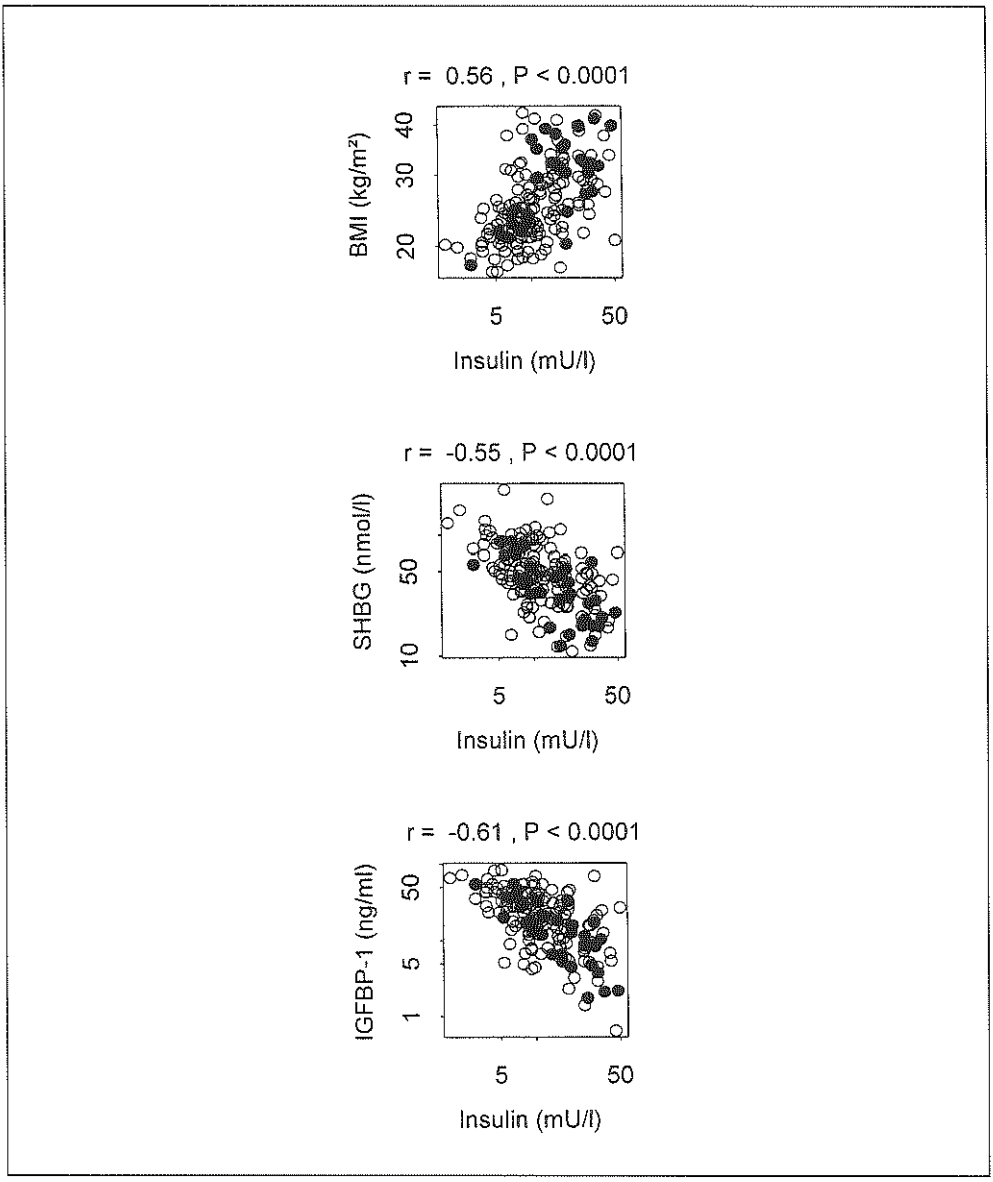

Figure 3 *Correlations between serum insulin and BMI, SHBG, and serum IGFBP-1 in 182 normogonadotropic oligoamenorrheic infertile patients using scatter plot. Open circles represent patients who ovulated following CC, and closed circles CC-non responders.*

The results of this study suggest that circulating inhibin B does not correspond to the number of healthy follicles present in polycystic ovaries, characterized by follicle maturation arrest and atresia of a proportion of follicles. This is in contrast with the conclusion of a recent report focusing on a relatively small group of patients (Anderson *et al.*, 1998). Recent observations suggest that increased intraovarian concentrations of VEGF may be related to increased stroma echogenicity and vascularity and increased secretion and pulsatility of LH (Agrawal *et al.*, 1998b). We have not been able to confirm that VEGF levels may represent the magnitude of ovarian dysfunction in these women.

The impact of markers for insulin sensitivity, the IGF system, leptin, inhibin B, and VEGF on the prediction of conception after ovulatory CC cycles has also been analyzed. None of these parameters predicts the probability of conceiving in women who ovulated after CC (data not shown). This stresses again the concept previously put forward (Imani *et al.*, 1999) that follicle growth and oocyte quality are differentially regulated.

In conclusion, the present longitudinal follow-up study suggests that FAI, as a marker for the severity of hyperandrogenemia, is the most significant endocrine marker of ovarian dysfunction and for the prediction of ovarian response after CC medication. Initial serum leptin, insulin, and IGFBP-1 levels are factors in univariate analyses predicting patients who will remain anovulatory after CC. The capability of serum insulin and IGFBP-1 to predict CRA disappears when FAI enters into the model due to a significant correlation between FAI and these endocrine parameters. Finally, leptin is a better marker to predict ovarian response to CC medication as compared to BMI. For the assessment of markers useful for clinical screening in patients, the present observations predicting ovarian response should be combined with factors predicting pregnancies in ovulatory CC women. Moreover, the question arises of whether factors involved in ovarian response or conception after CC medication may also predict individual response to exogenous gonadotropins in patients who fail to ovulate or conceive after CC.

2.5 A nomogram to predict live birth probability after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility

2.5.1 Introduction

Anovulation represents the most frequent cause of female infertility, and in most women normal serum follicle-stimulating hormone concentrations are found. Since its introduction (Greenblatt *et al.*, 1961) clomiphene citrate (CC) has been used worldwide as first choice medication in the treatment of anovulatory infertility. Since the early seventies CC treatment was restricted to normogonadotropic oligoamenorrheic infertility (WHO group 2) (World Health Organization. *et al.*, 1993). A significant proportion of these women, however, remains anovulatory after CC medication. Out of the 75% ovulatory CC patients, approximately 50% will conceive within 6 CC-induced cycles (Imani *et al.*, 1998; Imani *et al.*, 1999).

Assessment of pregnancy chances after CC induction of ovulation has been the subject of several investigations, all of which focused on the entire group of anovulatory patients who start with CC therapy (MacGregor *et al.*, 1968; Gorlitsky *et al.*, 1978; Shepard *et al.*, 1979; Hammond *et al.*, 1983; Polson *et al.*, 1989; Opsahl *et al.*, 1996; Kousta *et al.*, 1997). These investigators have failed to identify predictors of CC treatment outcome. In contrast, our group focused on prediction of ovulation and conception separately (Imani *et al.*, 1998; Imani *et al.*, 1999). This approach in life table analysis for prediction of pregnancy chances seems mandatory for the following statistical reasons. Discontinuation of CC therapy due to persistent anovulation is clearly an informative selective drop-out since chances for CC treatment outcome are different compared to women who continue CC therapy. Therefore, inclusion of both CC responders and CC non-responders in a life table analysis for prediction of conception should be avoided.

We developed two distinct prediction models applying multivariate analyses (Imani *et al.*, 1998; Imani *et al.*, 1999). The first model predicts ovarian response after CC in the entire group of anovulatory patients on the basis of initial screening characteristics such as; free androgen index (testosterone/sex hormone-binding globulin ratio), body mass index (BMI), cycle history (oligomenorrhea versus amenorrhea) and mean ovarian volume (Imani *et al.*, 1998; Imani *et al.*, 2000). The second model predicts the chances of conception exclusively in those women who have reached ovulatory cycles after CC and includes woman's age and cycle history (Imani *et al.*, 1999). Although scientifically sound, this approach seems difficult to apply in the daily clinical practice. Moreover, the question remains unanswered whether the chances of having a live birth after CC can be predicted prior to the

initiation of medication. By combining both prediction models a nomogram may be developed predicting chances of a given anovulatory patient to conceive resulting in live birth after CC.

Anovulatory patients present with a wide range of chances to conceive after CC. This may be due to differences in the underlying ovarian abnormalities, patient's age, body weight, and individual differences in the anti-estrogenic effects of CC on cervical mucus or endometrium. Applying a nomogram in the clinic may render the ovulation induction protocols more patient tailored and more cost-effective. Patients with a poor predicted chance to conceive could be advised to refrain from CC therapy and start with an alternative first line treatment modality such as weight reduction, insulin sensitizing agents, or exogenous gonadotropins. Particularly in women of advanced age precious time to ascertain that CC treatment is ineffective may be used for a more effective approach as the first line therapy. We now report the construction of a nomogram to identify characteristics upon initial screening of a large cohort of normogonadotropic anovulatory infertile women predicting the individual chance of pregnancy leading to live birth after CC induction of ovulation.

2.5.2 Materials and Methods

Subjects and study protocol

Between February 1993 and May 1999, two hundred and fifty nine patients attending our infertility unit were included in the present study using the following inclusion criteria: (a) oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval > 6 months), (b) serum follicle-stimulating hormone (FSH) levels within normal limits (1-10 IU/L) (World Health Organization. *et al.*, 1993; van Santbrink *et al.*, 1995), (c) normal serum prolactin and thyroid-stimulating hormone levels, (d) spontaneous menses or positive bleeding response to progestagen withdrawal, (e) body mass index (BMI) (weight divided by the square of the patients height) > 18 kg/m^2, (f) between 19-40 years of age, (g) no previous use of ovulation induction agents, (h) a total motile sperm count [TMC = ejaculate volume (milliliters) x sperm concentration (10^6 / mL) x percentage of progressive motile sperm] of the partner above 1 million, (i) negative history of any tubal pathology, and finally (j) no indication for intrauterine insemination. Institutional Review Board approval was obtained from the human subjects committee of the Erasmus University Medical Center Rotterdam and informed consent was obtained from all subjects.

Standardized initial clinical, sonographic, and endocrine screening took place prior to initiation of CC ovulation induction, as described previously (Imani *et al.*,

1998; Imani *et al.*, 1999; Imani *et al.*, 2000). Clinical screening included age, type of infertility, cycle history, BMI, waist-to-hip ratio (WHR), and previous medication and/or surgery. Transvaginal sonography (TVS) included assessment of the ovarian stroma echogenicity (arbitrarily classified from 1 to 3 per ovary), ovarian volume (mL) and total number of follicles (both ovaries), as described previously (Pache *et al.*, 1992; van Santbrink *et al.*, 1997b). Sonographic monitoring was performed by a single observer (B.I.). Endocrine screening included serum assays for FSH, luteinising hormone (LH), estradiol (E_2), androstenedione (AD), testosterone (T), and sex-hormone binding globulin (SHBG), fasting insulin and glucose, free and total insulin-like growth factor-I (IGF-I), inhibin B and leptin concentrations, as described previously (Imani *et al.*, 2000). Hormone assays used and the intra and inter assay coefficients of variation valid for this study have all been described previously (Imani *et al.*, 1998; Imani *et al.*, 1999; Imani *et al.*, 2000).

The treatment protocol, assessment of ovarian response and conception after CC have also been described previously (Imani *et al.*, 1998; Imani *et al.*, 1999). In brief, initial CC doses were 50 mg/day starting on cycle day 3 after a spontaneous or progestagen-induced withdrawal bleeding. In case of absent ovarian response, doses were increased to 100 and 150 mg / day in subsequent cycles. Ovulation after CC medication was assessed by sonographic monitoring of follicle growth and midluteal progesterone > 25 nmol/L. Conception was defined as a positive urinary pregnancy test (clearview, hCG II, Unipath Ltd, Bedford, UK) more than 3 days after the expected menses. Live birth was defined as delivery of a baby. Information regarding deliveries and the health condition of the babies born was collected using the hospital records. In the case of home delivery we collected information directly from the patient and her general practitioner or midwife.

Data analysis

The statistical analysis of conception leading to live birth in anovulatory patients after CC should take into account the following two steps: ovulatory response to CC and conception in case of ovulation. Patients remaining anovulatory after CC (CRA = clomiphene resistant anovulation) are considered to have no chance to conceive with this therapy. Therefore, the cumulative rate of conception leading to live birth was calculated by multiplying the chance of achieving ovulation after CC, with the estimated Kaplan-Meier (Kaplan *et al.*, 1958) cumulative probabilities for conception in the group of ovulatory women after CC.

A prediction model of the probability of conception resulting in live birth within 6 months after initiation of CC ovulation induction was constructed by combining the previously published prediction model for the chance to be CRA (Imani *et al.*, 1998) with a prediction model for the probability of conception leading to live

birth in ovulatory patients after CC. The current study differs from the previously published model predicting chances for conception (Imani *et al.*, 1999) with respect to the starting point of follow-up (initiation of CC therapy versus first ovulation after CC). In order to implement these findings in clinical practice we included months rather than ovulatory cycles and live birth instead of ongoing pregnancy in the current analysis. A nomogram was constructed by combining the model to predict ovulation in the entire group of women with the model predicting live birth in ovulatory CC patients.

To determine the goodness-of-fit of the combined prediction model, patients were divided into 5 equal groups according to quintiles of the predicted probability of conception leading to live birth. Using a Chi-square test, the mean predicted probability within each group was compared to the observed probability, calculated by the Kaplan-Meier method described in the previous paragraph. Data were analyzed using the commercially available software package SPSS (Chicago, Ill, USA).

2.5.3 Results

From the total number of 259 patients fulfilling the in/exclusion criteria and treated with CC, 186 (72%) suffered from primary infertility, and 62 (24%) from amenorrhea. One hundred and ten patients (43%) were obese (BMI > 27), 160 patients (62%) presented with an elevated FAI (FAI > 4.5) and 100 patients (39%) with hyperandrogenemia (T ≥ 3.2 nmol/L and/or AD ≥ 16.3 nmol/L (Fauser *et al.*, 1991; van Santbrink *et al.*, 1997b)). In 195 patients (75%) polycystic ovaries (mean ovarian volume ≥ 10.8 mL and/or mean follicle number per ovary ≥ 10 (Pache *et al.*, 1992; van Santbrink *et al.*, 1997b)) were diagnosed. One hundred and twenty-one patients (47%) presented with elevated initial LH (LH ≥ 7.0 IU/L (van Santbrink *et al.*, 1997b)) serum level. Sixty-five patients (25%) remained anovulatory after CC medication despite the maximum CC dose (150 mg/day) and were considered as CRA. Eighty-three patients (32%) presented with ovulatory cycles after CC but failed to conceive.

A total number of 111 (43%) women conceived after CC treatment and 11 (10% of conceptions) miscarried. There was one case of ectopic pregnancy and one case of intrauterine death. A total number of 98 (38%) patients had a live birth. Sixty-seven patients delivered at the hospital and could leave the unit within 24 hours. Nineteen patients were hospitalized for pre and postnatal care. In 4 pregnant women anti-hypertensive medication was required. There was no case of maternal or fetal death. In 15 women an instrumental delivery and in 13 patients a cesarean section was performed. At birth the mean ± SD gestational

age was 39.3 ± 2.8 weeks and the mean weight of the babies was 3142 ± 590 gram. There was no case of postpartum death in the first year after the delivery. No case of congenital abnormalities was reported.

Odds ratios of screening characteristics separately for ovulation in the entire population and live birth in ovulatory CC women are presented in Table 1. Screening characteristics were included on the basis of significance in previous multivariate analysis predicting ovulation

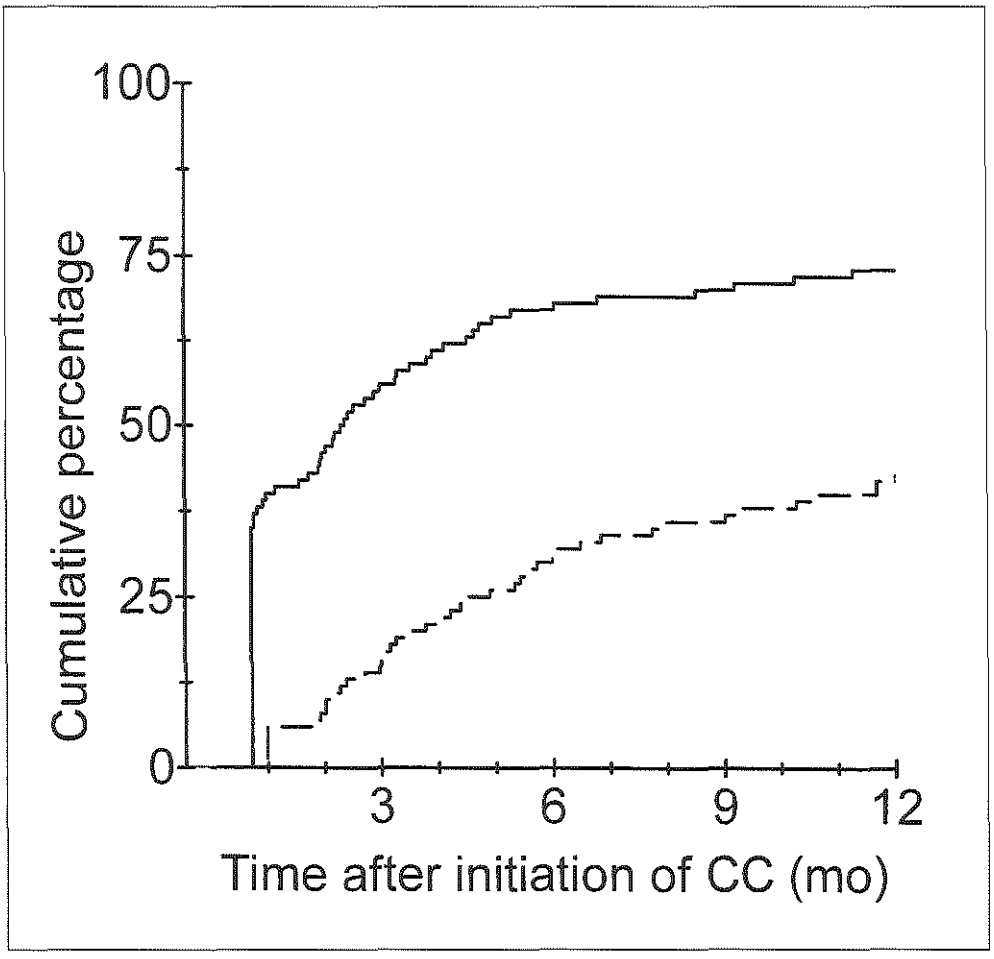

Figure 1 *Cumulative percentage of women reaching first ovulation and first conception ending in a live birth in 259 normogonadotropic oligoamenorrheic infertile patients treated with CC. Solid line = first ovulation after CC (n=194; 75%); dashed line = first conception ending in a live birth (n=98; 38%).*

TABLE 1. Odds ratios (OR) of initial screening characteristics in relation to the occurrence of ovulation in the entire group of normogonadotropic oligoamenorrheic infertile women (n=194 of 259) or live birth in ovulatory women after CC (n=98 of 194)

Screening characteristics	Ovulation following CC	Live birth in ovulatory women following CC
Clinical		
Age (per yr) [a]	1.05 (0.98 – 1.12)	0.90 (0.85 – 0.94)
Primary vs. secondary infertility [b]	1.23 (0.64 – 2.34)	0.71 (0.44 – 1.15)
Oligomenorrhea vs. amenorrhea [a, c, d]	4.34 (2.34 – 8.05)	0.77 (0.47 – 1.28)
Bleeding interval in case of oligomenorrhea [b, e]		
5-6 weeks	8.30 (3.29 –20.98)	0.65 (0.36 – 1.18)
6-9 weeks	3.64 (1.75 – 7.55)	0.87 (0.50 – 1.51)
9-26 weeks	2.90 (1.25 – 6.73)	0.83 (0.42 – 1.63)
BMI (kg/m^2) [c]	0.92 (0.88 – 0.96)	1.00 (0.97 – 1.04)
Waist to hip ratio (WHR per 0.1)[b]	0.60 (0.40 – 0.89)	1.08 (0.81 – 1.42)
Endocrine		
LH (IU/L)[b]	0.97 (0.91 – 1.03)	1.04 (1.00 – 1.09)
FAI [b, f]	0.83 (0.78 – 0.89)	1.06 (1.01 – 1.11)
Hyperandrogenemia (elevated T and / or AD) [b]	0.36 (0.20 – 0.65)	1.67 (1.10 – 2.54)
Insulin (mU/L) [b]	0.95 (0.92 – 0.98)	0.99 (0.97 – 1.02)
Insulin/glucose ratio [b]	0.87 (0.77 – 0.97)	0.99 (0.90 – 1.08)
Inhibin B (ng/L) [b]	1.00 (0.99 – 1.01)	1.00 (0.99 – 1.01)
Free IGF-I (ng/mL) [b]	0.91 (0.77 – 1.09)	1.10 (0.96 – 1.25)
Leptin (ng/mL) [c]	0.97 (0.95 – 0.99)	1.02 (1.00 – 1.03)
Ultrasound		
PCO versus non-PCO [c, g]	0.59 (0.29 – 1.22)	0.98 (0.63 – 1.54)
Sperm parameters		
Total motile sperm count / 10^4 [b]	-	1.00 (0.81 – 1.23)

[a] Characteristics predicting conception in ovulatory CC patients applying multivariate analysis as published previously (Imani et al, JCEM 1999)
[b] Biological relevant characteristics for ovarian dysfunction or pregnancy prediction in normogonadotropic anovulation
[c] Characteristics predicting ovulation following CC applying multivariate analysis as published previously (Imani et al, JCEM 1998, JCEM 2000)
[d] The oligomenorrheic patients are 4 times more likely (OR = 4.34) to ovulate after CC compared to amenorrheic patients. However the chance for a live birth after CC is 23% less (OR = 0.77) compared to amenorrheic patients.
[e] Amenorrhea is reference category for the categories of bleeding interval in oligomenorrhea.
[f] FAI = T x 100 / SHBG
[g] Polycystic ovaries = mean ovarian volume ≥ 10.8 mL and/or mean follicle number per ovary ≥ 10 (van Stanbrink et al, Fertil Steril 1997)

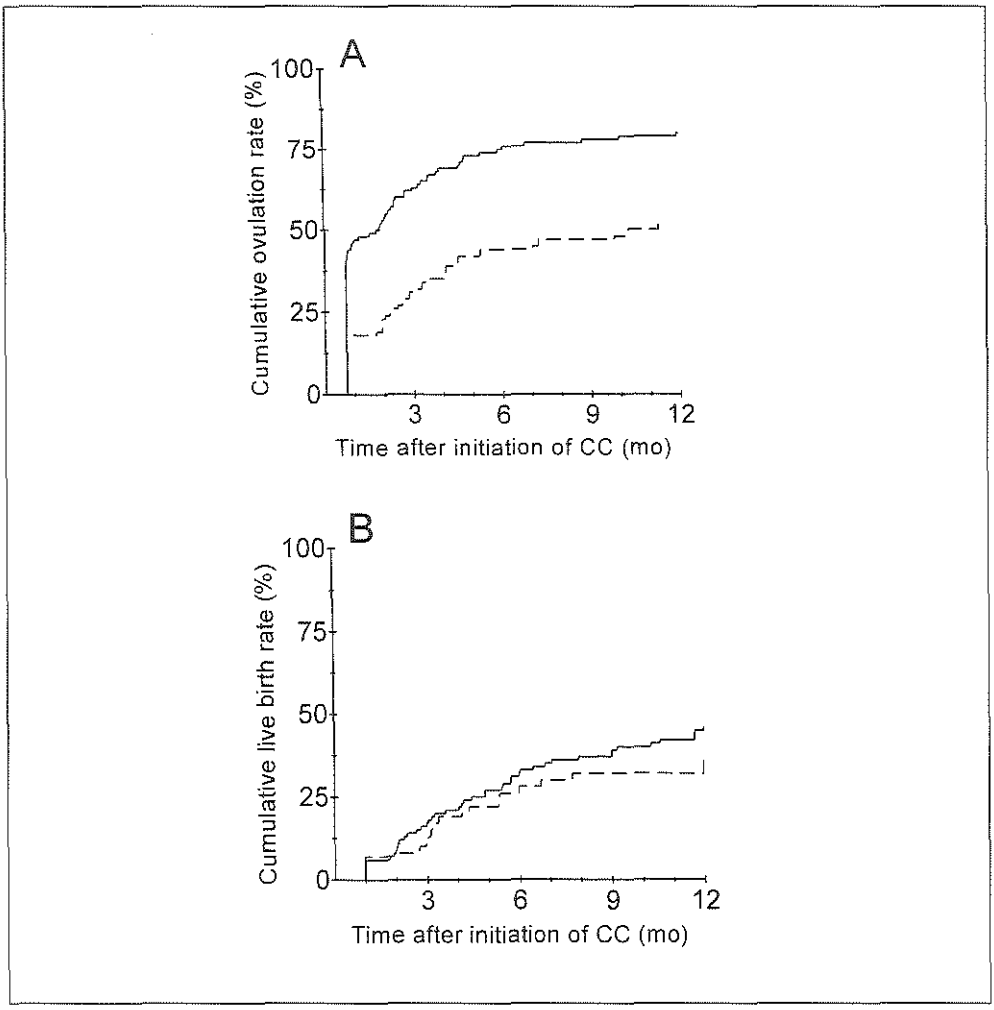

Figure 2 *The impact of cycle history on cumulative ovulation and live birth rates in 259 normogonadotropic oligoamenorrheic infertile patients treated with CC. A: solid line = oligomenorrheic patients (n = 197); B: dashed line = amenorrheic patients (n = 62). P value represents differences applying LogRank test (A: P < 0.001; B: P value = not applicable in the overall WHO 2 anovulatory patients. P < 0.001 in ovulatory patients following CC).*

(FAI, BMI, cycle history, ovarian volume, leptin), predicting pregnancy in ovulatory patients (age, cycle history) and biological relevance for ovarian dysfunction (LH, hyperandrogenemia, insulin, insulin/glucose ratio, inhibin B, free IGF-I) or pregnancy prediction (infertility history, bleeding interval in case of oligomenorrhea, total motile sperm count). The cumulative rates of first ovulation and first conception ending in live birth are depicted in Figure 1. The impact of cycle his-

tory on cumulative ovulation and live birth rates after CC is depicted in Figure 2.

Prediction of conception leading to live birth after CC applying a nomogram has been depicted in Figure 3. This nomogram comprises two separate steps. In the left panel the probability of ovulation after CC can be estimated applying FAI, BMI, and cycle history (oligomenorrhea versus amenorrhea). Drag and drop the chance to ovulate after CC from the left to the right panel. In the right panel the predicted probability to conceive within 6 months after initiation of CC medication resulting in live birth is indicated, combining the probability of ovulation after CC with age and cycle history (oligomenorrhea versus amenorrhea) of the patient. Mean ovarian volume, and serum leptin levels comprise the least predictive contribution to the model (Imani *et al.*, 1998; Imani *et al.*, 2000) and therefore were excluded from the left panel of this nomogram.

The P-values for the Chi-square goodness-of-fit test was 0.49, indicating no statistically significant lack of fit between the observed and predicted probability of live birth within the 5 groups (see also Materials & Methods; data not shown).

2.5.4 Discussion

Currently, CC represents the first line treatment strategy for all patients with normogonadotropic anovulatory infertility. The present study confirms that CC is safe and convenient with limited chances for complications such as multiple pregnancy or OHSS. However, only 38% of treated women may conceive after CC resulting in live birth which is substantially less than generally assumed. It may represent a distinct step forward if patients with even lower chances for live birth after CC could be identified in advance.

Previous attempts by retrospective studies in the entire group of anovulatory patients failed to identify factors predicting ovulation (Gorlitsky *et al.*, 1978; Shepard *et al.*, 1979; Polson *et al.*, 1989) or conception (Gorlitsky *et al.*, 1978; Hammond *et al.*, 1983) (Kousta *et al.*, 1997) after CC. In contrast, we focused on predictors of CC treatment outcome in a prospective fashion in the current longitudinal cohort study. For the first time we used ovulation and conception as separate endpoints, taking into account that a significant proportion of patients remains anovulatory during CC medication. These women are not exposed to the chances of becoming pregnant. Univariate and multivariate analyses were performed in these two separate settings. The first multivariate model consisting of FAI, BMI, cycle history, and mean ovarian volume predicts chances of remaining anovulatory after CC (Imani *et al.*, 1998). The second model consisting of age and cycle history predicts chances for conception only in women who respond to CC

(Imani *et al.*, 1999). Observed screening characteristics involved in the prediction of ovulation after CC medication are distinctly different from predictors of conception in ovulatory CC patients.

In the current study we combine both prediction models in a nomogram to predict chances of live birth in the entire group of normogonadotropic anovulatory patients. For reasons of its applicability in clinical practice we converted analysis to month rather than treatment cycles and used live birth rather than pregnancy as the outcome. The estimation of chances to ovulate is a crucial step in evaluating the probability of conception resulting in live birth after CC therapy. This is the reason for the involvement of two steps in the presented nomogram. Cycle history is the only factor which is present in both steps. Amenorrheic patients are less likely to ovulate after CC medication, but once they ovulate chances for live birth are higher compared to oligomenorrheic women. A biologically plausible explanation is that the occurrence of other factors contributing to a reduced fertility is less likely since these women never had the chance to conceive. BMI does not influence live birth rate in patients who ovulate following CC. Moreover, live birth rate in CC-ovulatory PCO *vs.* CC-ovulatory non-PCO patients are comparable. Interestingly, age does not affect chances for ovulation, but is involved in pregnancy chances once ovulation has been reached (Imani *et al.*, 1998; Imani *et al.*, 1999). This is in agreement with reports concerning an association between reduced treatment outcome after *in vitro* fertilization and patient's advanced age (Scott *et al.*, 1995).

The major aim was to render the present nomogram as easy as possible for use in daily clinical practice. Therefore, ovarian volume (which was included in the previous multivariate model predicting ovulation (Imani *et al.*, 1998)) has been excluded from the present nomogram. The additional predictive contribution of this prognostic factor to the model is negligible (a decrease in the area under the receiver characteristic curve of the model to predict patients remaining anovulatory from 0.82 to 0.80 (Imani *et al.*, 1998)). We also demonstrated previously that serum leptin concentrations (an endocrine marker associated with obesity (Rouru *et al.*, 1997)) predict chances of remaining anovulatory after CC (Imani *et al.*, 2000). Leptin has also been excluded from the present nomogram since this assay is not available in most hospital laboratories. Instead, information for BMI can be generated easily with only a minor decrease in AUC of the prediction model (0.85 instead of 0.82). Therefore, serum T and SHBG levels are the only endocrine factors which should be assessed for the present nomogram. Indeed, both obesity and hyperandrogenemia are associated with polycystic ovary syndrome known to exhibit low chances for success during CC ovulation induction. Remaining biological plausible factors involved in ovarian dysfunction in normogonadotropic

anovulation such as LH (Fauser *et al.*, 1991), insulin/glucose ratio's (Poretsky *et al.*, 1999), inhibin B (Anderson *et al.*, 1998) and free IGF-I (van Dessel *et al.*, 1999) failed to predict ovulation or conception and were therefore excluded from the nomogram.

In summary, the present study demonstrates for the first time that a nomogram can be developed on the basis of initial screening characteristics predicting chances of live birth after CC for a given patient. An external validation of the present nomogram is mandatory to be able to define a clear cut-off level in the chances for live birth after CC for the decision making in routine daily clinical practice. For instance, a cut-off level of 20% chance of having a live birth (which comprises 19% of the overall WHO 2 anovulatory patients) could be chosen. These patients could be advised to refrain from CC therapy and start with an alternative first line treatment modality such as weight reduction, exogenous gonadotropins, insulin sensitizing hormones, or *in vitro* fertilization particularly in women with an advanced age. This would render CC ovulation induction strategies more patient tailored and could improve overall cost effectiveness.

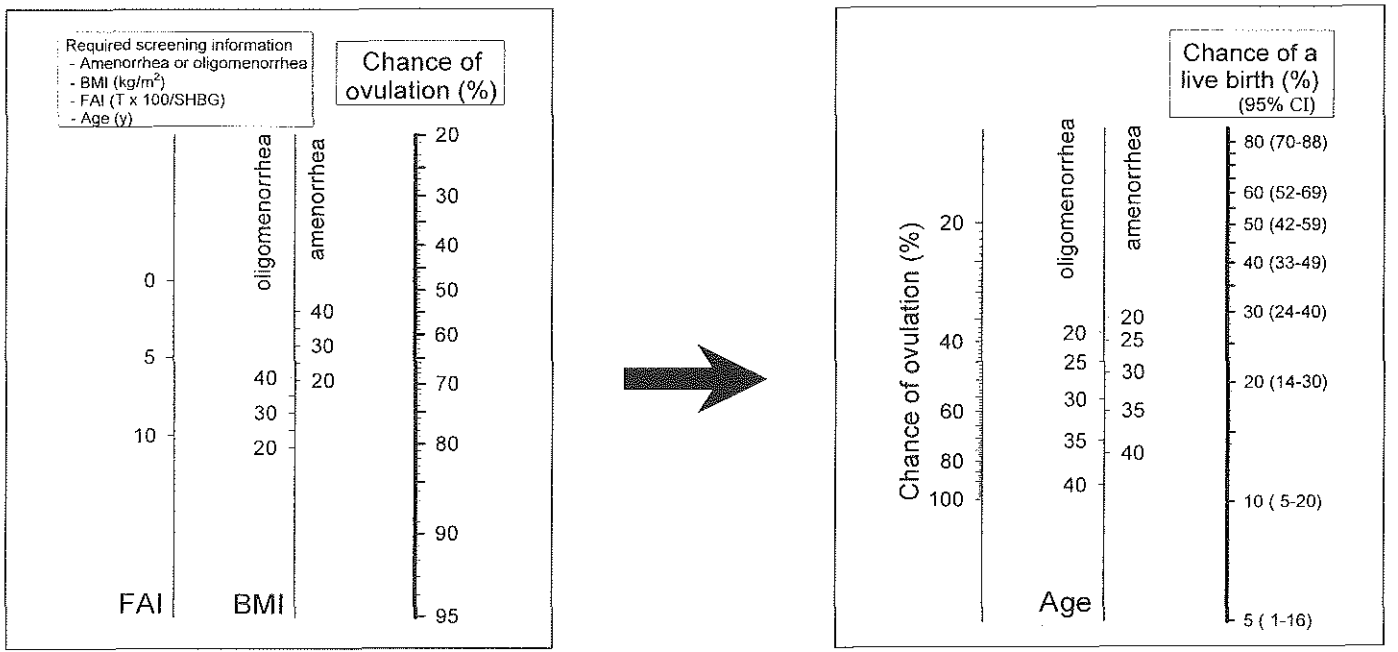

Figure 3 *The probability to ovulate and conceive ending in a live birth within 6 months after initiation of CC therapy applying a nomogram. Chances for ovulation following CC can be assessed using FAI, BMI, and cycle history (left panel). The probability to conceive ending in a live birth within 6 months after initiation of CC can be assessed by combining the probability to ovulate with the age and the cycle history of the woman (right panel). Encircle the values related to each screening parameter. Connect the circles to observe the predicted probability to ovulate. Correspond this probability from the left to the right panel and connect it with the age of the woman (separately for oligomenorrhea or amenorrhea) to predict the chance of a live birth. For instance, a 29 year old amenorrheic patient presented with the following findings: FAI = 9.3, BMI = 32. Her chance to conceive leading to a live birth within 6 months after initiation of CC medication will be 19% (chance to ovulate following CC: 50%).*

Chapter III

Prediction of exogenous gonadotropin ovaluation induction outcome

3.1 Introduction

As indicated before, complications of induction of ovulation applying exogenous gonadotropins in the group of normogonadotropic clomiphene resistent oligomenorrheic or amenorrheic infertile women are a cause of major concern. These complication are mainly OHSS and multiple pregnancy, which seems to be correlated with the dose regimens applied (van Santbrink *et al.*, 1997a), late follicular phase FSH accumulation and / or multifollicular development (Fauser *et al.*, 1997). Moreover, a large variability exists in the amount of exogenous FSH required due to individual differences in ovarian sensitivity for FSH stimulation - also referred to as the FSH threshold (Brown *et al.*, 1978) - of these women. This has been held responsible for the high incidence of complications resulting from the development of multiple dominant follicles which may result in multiple pregnancies and sometimes OHSS (Gleicher *et al.*, 2000).

In an attempt to reduce these complications alternative gonadotropin treatment schedules have been developed. These treatment protocols have been described in chapter 1.2.2 and 1.3.2. We introduced a safe and effective protocol to treat a new clomiphene resistent patients applying exogenous gonadotropins for induction of ovulation. An initial dose-finding cycle according to a low-dose step-up protocol prior to subsequent step-down cycles was applied to identify patients with extreme low or high FSH threshold. The starting does of FSH in the subsequent cycle was initiated according to a step-down dose regimen with initial starting dose of $^1/_2$ ampoule above the effective dose (FSH response dose) in the preceding low-dose step-up cycle.

Prediction of FSH threhold (FSH response dose) in normogonadotropic clomiphene resistent oligomenorrheic or amenorrheic infertile women has been the focus of the first part of the present chapter. Subsequently, in the last part of the present chapter we report on prediction of multifollicular development, conception during exogenous gonadotropins for induction of ovulation applying step-down dose regimen.

3.2 Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in normogonadotropic anovulatory infertility: An approach to increase safety and efficiency

3.2.1 Introduction

Chronic anovulation is a common cause of infertility in women, the majority of whom have irregular menstrual cycles and normal serum FSH concentrations

(World Health Organization [WHO], group 2) (World Health Organization. *et al.*, 1993). Approximately 60% of these women are diagnosed as polycystic ovary syndrome (PCOS), dependent on inclusion criteria used (van Santbrink *et al.*, 1997b). Treatment of first choice in WHO 2 is anti-estrogen medication, commonly clomiphene citrate (CC) (Franks *et al.*, 1995). Approximately 50% of WHO group 2 anovulatory women who have failed to ovulate or conceive after CC medication can be successfully treated with exogenous gonadotropins (Franks *et al.*, 1995; Fauser *et al.*, 1997). Currently, low-dose step-up (White *et al.*, 1996) or step-down (van Santbrink *et al.*, 1995; van Santbrink *et al.*, 1997a) regimens are used to reduce the probability of multiple pregnancies and ovarian hyperstimulation syndrome (OHSS) compared to initially introduced high dose gonadotropin protocols (Fauser *et al.*, 1997).

Exogenous gonadotropins elicit significantly increased ovulation rates (up to 90%) compared to CC (White *et al.*, 1996; Imani *et al.*, 1998) and result in similar conception rates (55%) in case ovulatory cycles are obtained (van Santbrink *et al.*, 1995; White *et al.*, 1996; Imani *et al.*, 1999). However, a large variability exists in the amount of exogenous FSH required due to individual differences in ovarian sensitivity for FSH stimulation - also referred to as the FSH threshold (Brown *et al.*, 1978) - of these women. This has been held responsible for the high incidence of complications resulting from the development of multiple dominant follicles which may result in multiple pregnancies and sometimes OHSS (Gleicher *et al.*, 2000). The increased incidence of multiple gestation has significant social, economic, and health consequences (Callahan *et al.*, 1994).

The narrow range of FSH serum concentrations resulting in either mono or multi-follicular growth has been a major concern. Thus, intense monitoring of patients undergoing gonadotropin induction of ovulation is mandatory to adjust the FSH dose according to the individual ovarian response. One method of fine-tuning the required FSH levels and reducing complications is by starting with a low dose of FSH and gradually increasing the dose until ovarian response is observed (White *et al.*, 1996; Fauser *et al.*, 1997), a protocol currently referred to as the "low-dose, step-up" regimen. However, this can be a time-consuming procedure, especially for patients who exhibit a high FSH threshold.

Another treatment strategy, developed by our group, is the "step-down" regimen (van Santbrink *et al.*, 1995; Fauser *et al.*, 1997; van Santbrink *et al.*, 1997a) which involves a relatively high initial dose of FSH followed by subsequent decremental steps. This approach more closely mimics physiological conditions in normo-ovulatory women (van Santbrink *et al.*, 1995). However, the initial standard FSH dose may be too high for those women with a low FSH threshold level which could induce imminent ovarian hyperstimulation requiring cancellation of stimu-

lation (Fauser *et al.*, 1997). Adjusting the starting dose in women with a low FSH threshold would certainly reduce the incidence of multiple dominant follicle development and related complications during the step-down regimen.

The ability of clinicians to choose an appropriate starting dose of FSH for a given patient could considerably improve treatment outcome in both low-dose step-up or step-down regimens and render this treatment modality safer and more efficient. Clinicians, who prefer to use a low-dose step-up regimen, may start with a higher FSH starting dose in patients with a predicted high FSH threshold. These patients may reach adequate ovarian stimulation in a shorter period of time with fewer requirements for ovarian response monitoring and less gonadotropin preparations administered in doses below the FSH threshold. In contrast, women with a low predicted FSH threshold may start with lower initial dose of FSH to minimize complication or cancellation rates especially when a step-down protocol is applied. The present study was designed to develop a model, which uses pre-treatment clinical, endocrine, and sonographic screening characteristics of normogonadotropic anovulatory women who did not ovulate or conceive after preceding CC medication, to predict the individual FSH response dose for gonadotropin induction of ovulation.

3.2.2 Materials and Methods

Subjects and study design

Ninety women attending our infertility unit between June 1997 and September 1999 were included in the present study, using the following criteria; (a) oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval > 6 months), (b) serum FSH levels within normal limits (1-10 IU/L), (van Santbrink *et al.*, 1995; Schipper *et al.*, 1998) (c) normal serum prolactin and thyroid-stimulating hormone levels, (d) spontaneous menses or positive bleeding response to progestagen withdrawal, (e) body mass index (BMI) (weight divided by square height) > 18 kg/m^2, (f) age between 19-40 years, and (g) previously being treated unsuccessfully with CC (failure to ovulate, or failure to conceive in six ovulatory CC-cycles) in our fertility unit. Institutional Review Board approval was obtained from the human subjects committee of the Erasmus University Medical Center Rotterdam and informed consent was obtained from all subjects.

Standardized initial clinical, sonographic, and endocrine screening (as described previously (Imani *et al.*, 1998; Imani *et al.*, 1999)) took place prior to initiation of exogenous gonadotropin induction of ovulation using a low-dose step-up regimen during the first stimulation cycle. Clinical screening included age, duration and history (primary *vs.* secondary) of infertility, cycle history, ovarian

response to previous CC medication, BMI, waist-to-hip ratio (WHR), any other previous medication and/or surgery. Transvaginal sonographic (TVS) screening included assessment of the ovarian stroma echogenicity (arbitrarily classified from 1 to 3 per ovary), ovarian volume (mL) and total number of follicles (both ovaries), as described previously (Pache *et al.*, 1992; van Santbrink *et al.*, 1997b). Sonographic monitoring was performed by a single observer (B.I.), using an ultrasound machine (model EUB-415, Hitachi Medical Corp., Tokyo, Japan) with a 6.5-MHZ transvaginal transducer. Endocrine screening included serum assays for FSH, luteinizing hormone (LH), estradiol (E_2), androstenedione (AD), testosterone (T), and sex-hormone binding globulin (SHBG), fasting insulin and glucose, free and total insulin-like growth factor-I (IGF-I), IGF-binding protein-1 (IGFBP-1), IGFBP-3, inhibin B and leptin concentrations, as described previously (Imani *et al.*, 2000).

Hormone assays used for this study have all been described previously (Imani *et al.*, 1998; Imani *et al.*, 2000). Intra- and interassay coefficients of variation were less than 6.1% and 9.9% for insulin, less than 1.0% and 1.9% for glucose, less than 10.3% and 10.7% for free IGF-I, less than 3.4% and 8.2% for IGF-I, less than 5.2% and 6.0% for IGFBP-1, less than 3.9% and 1.9% for IGFBP-3, less than 8% and 14% for inhibin B, and less than 3.6% and 4.6% for leptin, respectively.

Treatment protocol and monitoring of ovarian response

A low-dose step-up regimen using either daily intra-muscular (IM) injections of urinary FSH (Metrodin HP®, Serono Benelux BV, The Hague, The Netherlands) or subcutaneous (SC) injections of recombinant FSH (Gonal-F®, Serono Benelux BV) was applied starting on day 3-5 after a spontaneous or progestagen-induced withdrawal bleeding. The starting dose of FSH was one 75 IU per day. The first increase in the FSH dose by 38 IU per day was based on absence of ovarian response (i.e. sonographic visualization of a follicle ≥ 10 mm (Pache *et al.*, 1990; van Santbrink *et al.*, 1995)) after 7 days of medication. The FSH dose was kept constant for the following seven days and subsequently increased by 38 IU per day in case ovarian response was still lacking. Monitoring was performed every other day. In case of sufficient ovarian response, the FSH dose remained unaltered until administration of human chorionic gonadotropin (hCG) (Pregnyl®, NV Organon, Oss, The Netherlands). A single dose of 5,000 IU of hCG was administered IM on the day one or two follicles ≥ 18 mm were visualized. In case three or more follicles ≥ 16 mm were present, stimulation was cancelled and hCG withheld. Ovulation was assessed after hCG administration by the sonographic visualization of collapse of the dominant follicle and mid-luteal progesterone (P) levels above 25 nmol/l. No luteal support was provided.

Data analysis

All patients were followed during the first gonadotropin-induced cycle. We used the F test for ANOVA and Chi-square test for comparison of FSH response dose between variables. To assess the univariable relation between the initial screening parameters and FSH response dose, Pearson's correlation coefficients were computed. Multiple linear regression was used for multivariable analysis, with FSH response dose as a continuous outcome variable. Initial screening parameters significant in the univariable analysis, were entered into the multiple regression model in a forward stepwise fashion.

The overall predictive performance of the final model was assessed by multiple R^2. This number expresses the fraction of the total variance of FSH response dose that is explained by the regression model. The average error of the model when used for prediction of the FSH response dose of new patients was assessed by the Root Mean Squared Error (RMSE). R^2 and RMSE were computed on the same data that were used for developing the model. A bootstrapping procedure with 200 replications was therefore used to estimate the amount of over-optimism. From this, corrected R^2 and RMSE were calculated. $P = 0.05$ was deemed as threshold level for statistical significance. Data were analyzed using the commercially available software package SPSS (Chicago, IL, USA).

3.2.3 Results

Of the 90 women who failed to conceive after CC, 40 (44%) patients were considered to be CRA and the remaining 50 (56%) women were CC failures (CCF) (CC-induced ovulatory cycles without conception). During ovulation induction by exogenous FSH using a low-dose step-up regimen, all patients developed a follicle beyond 10-mm diameter and visualization of ongoing dominant follicle growth beyond 10 mm was observed at a dose of 75 IU/d in 33 (37%), 112.5 IU/d in 27 (30%), 150 IU/d in 16 (18%), 187.5 IU/d in 10 (11%) and > 187.5 IU/d in only 4 (4%) patients, respectively. A total number of 5 cycles (5.5%) have been cancelled due to multiple follicle development (> 3 follicle beyond 16 mm).

Distribution of CRA or CCF, and amenorrheic or oligomenorrheic patients related to the FSH dose administered on the day of ovarian response are depicted in Figure 1. Initial clinical, endocrine, and sonographic characteristics of the overall study group and separately for patients who reached an ovarian response at a daily dose of 75, 112.5, 150, or > 187.5 IU/day are presented in Table 1. Cycle history (amenorrhea or oligomenorrhea), bleeding interval (arbitrary classified in 4 categories), ovarian response to CC medication (CRA or CCF), BMI, WHR, T,

SHBG, free androgen index (FAI = 100 x T / SHBG), insulin, insulin/glucose ratio, free IGF-I, IGFBP-1, and leptin serum levels were all significantly different and correlated with the FSH response dose.

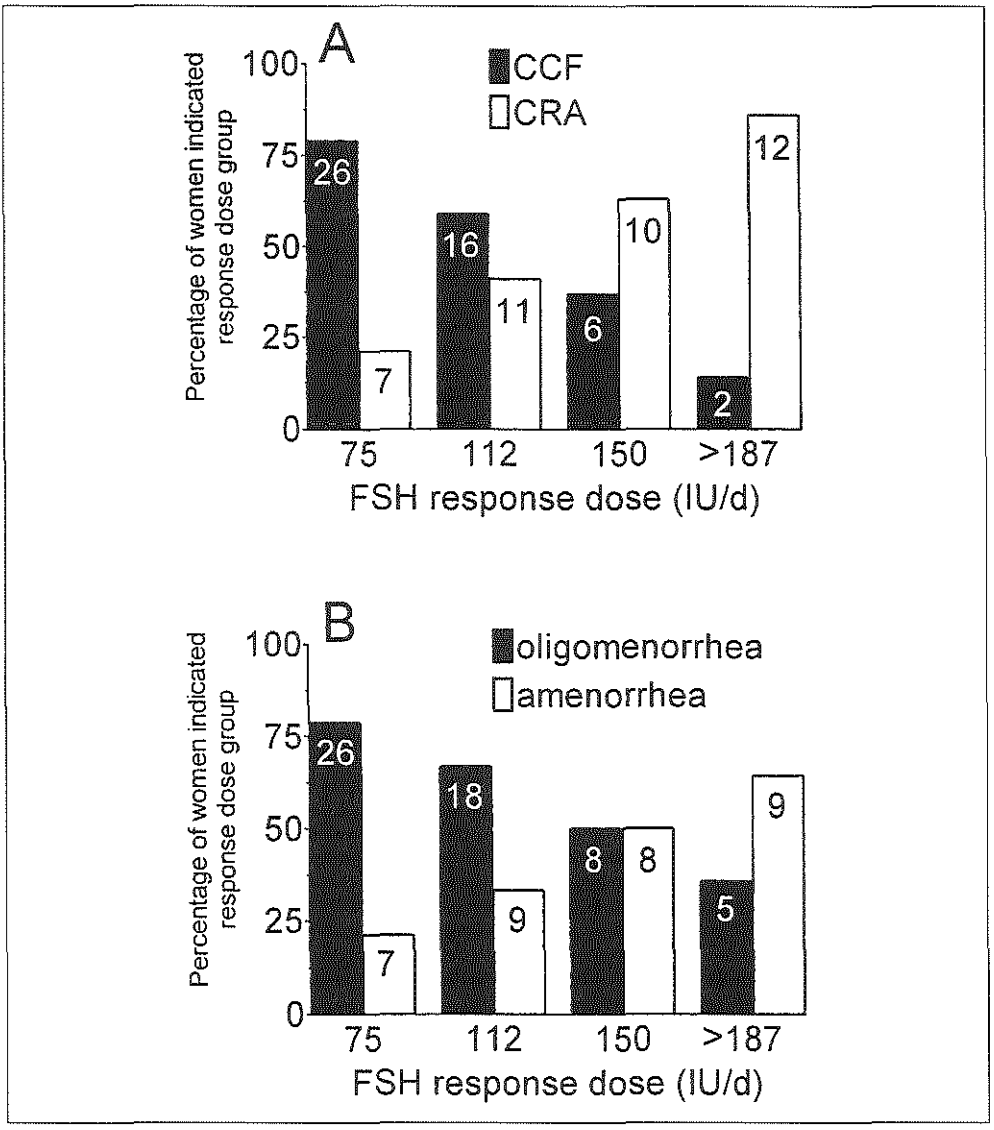

Figure 1 *Distribution of 90 normogonadotropic anovulatory infertile women in 4 different FSH response dose groups (75, 112.5, 150, or ≥ 187.5 IU/day) during ovulation induction by exogenous FSH using a low-dose step-up regimen. The following initial prognostic factors have been depicted: Ovarian response to the preceding CC medication (CRA or CCF; A) and cycle history (amenorrhea or oligomenorrhea; B). A: P < 0.001 (CCF vs. CRA). B: P <0.001 (oligomenorrhea vs. amenorrhea)*

The result of univariate and multivariate analysis for prediction of the FSH response dose is depicted in Table 2. The multiple R^2 of model step 1, 2, 3 were 0.29, 0.42, and 0.51 respectively. The multiple R^2 of the final model was 0.54, and the average prediction error (RMSE) was 30.6. The bootstrapping procedure revealed that the corrected values of R^2 and RMSE were 0.39 and 35.4, respectively. This means that we may expect the model to explain 39% of the between women variability in FSH response dose, and that the standard error of the predicted value is about 35 IU/L. The following equation for the predicted FSH response dose has been developed; [4 BMI (kg /m^2)] + [32 CRA (yes = 1 or no = 0)] + [7 free IGF-I (ng/mL)] + [6 FSH (IU/L)] - 51. The scatter plot between observed and predicted FSH response doses using this model is depicted in Figure 2. Box and Whisker plots of 2 initial screening parameters (significant at $P < 0.01$ in univariate analysis) of patients separated in four different groups of FSH response doses are depicted in Figure 3.

TABLE 1. Clinical, endocrine, and ultrasound characteristics (mean ± SD) during initial screening of 90 normogonadotropic anovulatory infertile women who did not ovulate or conceive after previous clomiphene citrate medication. Observations are also separated for patients who reached an ovarian response by the daily administration of 75, 112.5, 150, ≥ 187.5 IU exogenous FSH (FSH response dose) applying a low-dose step-up regimen for induction of ovulation.

	Overall group	FSH response dose				P^a
	n = 90	75 IU/d	112.5 IU/d	150 IU/d	≥ 187.5 IU/d	value
		37% (n = 33)	30% (n = 27)	18% (n = 16)	15% (n = 14)	
Screening parameters						
Clinical						
Age (years)	29 ± 4	29 ± 3	29 ± 4	29 ± 5	29 ± 3	–
Primary infertility % (n)	66% (59)	67% (22)	63% (17)	63% (10)	71% (10)	–
Bleeding interval in 4 categories					< 0.001[b]	
5-6 weeks, % (n)	19% (17)	36% (12)	11% (3)	6% (1)	7% (1)	
6-9 weeks, % (n)	28% (25)	30% (10)	37% (10)	19% (3)	14% (2)	
9-26 weeks, % (n)	16% (15)	12% (4)	19% (5)	25% (4)	14% (2)	
Amenorrhea, % (n)	37% (33)	21% (7)	33% (9)	50% (8)	64% (9)	
Ovarian response to CC (CRA)	44% (40)	21% (7)	41% (11)	63% (10)	86% (12)	< 0.001[b]
BMI (kg/m^2)	26.5 ± 5.5	23.0 ± 4.4	27.4 ± 4.5	28.8 ± 4.3	30.3 ± 6.6	< 0.001
Waist to hip ratio (WHR)	0.81 ± 0.08	0.78 ± 0.06	0.81 ± 0.08	0.87 ± 0.11	0.85 ± 0.06	0.003
Endocrine						
LH (IU/L)	8.1 ± 4.7	8.4 ± 5.1	7.3 ± 4.9	8.9 ± 4.2	8.1 ± 4.0	–
FSH (IU/L)	5.1 ± 1.5	4.8 ± 1.6	5.3 ± 1.4	5.1 ± 1.7	5.1 ± 1.6	–
E_2 (pmol/L)	236 ± 106	242 ± 130	213 ± 104	258 ± 71	242 ± 73	–
AD (nmol/L)	13.9 ± 6.7	12.9 ± 4.9	12.7 ± 4.8	16.4 ± 10.0	16.0 ± 8.4	–

T (nmol/L)	2.5 ± 1.0	2.4 ± 0.8	2.3 ± 0.9	3.1 ± 1.4	2.6 ± 0.7	0.024
SHBG (nmol/L)	48 ± 25	61 ± 26	43 ± 23	38 ± 22	39 ± 17	0.002
FAI (T x 100 / SHBG)	7.1 ± 5.4	4.7 ± 2.7	6.9 ± 5.2	11.5 ± 8.2	8.0 ± 3.5	< 0.001
Insulin (mU/L)	12.3 ± 8.2	8.7 ± 4.2	12.6 ± 5.7	15.6 ± 8.6	16.9 ± 14.6	0.006
Glucose (mmol/L)	4.3 ± 0.7	4.2 ± 0.8	4.0 ± 0.5	4.0 ± 0.9	4.4 ± 0.9	_
Insulin/glucose ratio	3.0 ± 2.1	2.1 ± 1.0	2.9 ± 1.3	4.2 ± 2.4	4.1 ± 3.6	0.002
Inhibin B (ng/L)	164 ± 134	196 ± 145	144 ± 126	115 ± 101	183 ± 148	_
IGF-I (ng/mL)	244 ± 80	258 ± 65	237 ± 88	238 ± 87	232 ± 92	_
Free IGF-I (ng/mL)	2.8 ± 1.7	2.6 ± 1.4	2.5 ± 1.4	2.8 ± 1.9	3.9 ± 2.4	0.09
IGFBP-1 (ng/mL)	24.3 ± 17.0	34.0 ± 17.7	21.7 ± 14.7	16.8 ± 14.2	15.8 ± 12.1	< 0.001
IGFBP-3 (ng/mL)	3,469 ± 757	3,498 ± 738	3,392 ± 776	3,513 ± 980	3,503 ± 434	_
Leptin (ng/mL)	20.0 ± 15.0	10.4 ± 5.0	22.3 ± 11.4	26.0 ± 16.9	31.3 ± 22.4	< 0.001
Ultrasound						
Total stroma score[c]	4.2 ± 1.2	4.0 ± 1.1	4.3 ± 1.3	4.8 ± 1.1	4.1 ± 1.4	_
Mean ovarian volume (mL)	10.7 ± 4.2	10.0 ± 3.6	9.9 ± 2.0	12.1 ± 5.4	12.4 ± 6.2	_
Mean follicle number	15 ± 6	14 ± 5	15 ± 5	18 ± 8	14 ± 8	_

[a] *P* value of F test for ANOVA for continuous variables, and *P* value of Chi-square test for categorical variables

[b] FSH response dose differs among four categories of bleeding interval

[c] Arbitrarily defined as one to three per ovary (both ovaries added)

TABLE 2. Forward stepwise multivariate analyses of initial screening parameters for prediction of the FSH response dose during FSH induction of ovulation applying a low-dose step-up regimen in 90 normogonadotropic anovulatory infertile women who failed to ovulate or conceive following CC

Analyses	Univariate [a]	Multivariate [b]			
Steps	0	1	2	3	4 [c]
Screening parameters					
Clinical					
Amenorrhea (n = 33) [d]	0.32 [e]	0.39 [e]	0.21	0.18	0.12
Ovarian response to CC medication (CRA)	0.49 [e]	0.43 [e]	In model	In model	In model
BMI (kg/m^2)	0.54 [e]	In model	In model	In model	In model
Endocrine					
FSH (IU/L)	0.06	0.19	0.30 [e]	0.27 [f]	In model
FAI (T x 100 / SHBG)	0.28 [f]	-0.02	-0.16	-0.16	-0.14
Insulin (mU/L)	0.35 [e]	0.14	0.11	0.09	0.10
Free IGF-I (ng/mL)	0.33 [e]	0.42 [e]	0.38 [e]	In model	In model
IGFBP-1 (ng/mL)	-0.36 [e]	-0.06	-0.06	-0.09	-0.10
Leptin (ng/mL)	0.53 [e]	0.25 [f]	0.20	0.11	0.13
Ultrasound					
Mean ovarian volume (mL)	0.17	0.14	0.02	-0.00	0.03

[a] Pearson's correlations (step 0, univariate). Only variables significant in univariate analysis were used in this analysis.

[b] Partial correlations, corrected for parameters entered into the model (step 1 - 4, multivariate)

[c] Final model, multiple $R^2 = 0.54$

[d] Amenorrhea was used as candidate variable instead of bleeding interval in 4 categories (as in Table 1) for ease of interpretation

[e] Significant at $P < 0.01$

[f] Significant at $P < 0.05$

Predicted FSH response dose calculated by a formula based on 4 selected variables in the final model: [4 BMI (kg /m^2)] + [32 CRA (yes = 1 or no = 0)] + [7 free IGF-I (ng/mL)] + [6 FSH (IU/L)] - 51. As an example, a new amenorrheic infertile patient who did not respond to CC medication presented with the following findings: BMI = 30.75, initial free IGF-I = 2.9 ng/ml, and initial serum FSH = 6.8 IU/l. The predicted FSH response dose is 123 + 32 + 20 + 41 − 51 = 165 IU / day which equals 2.2 ampoules / day.

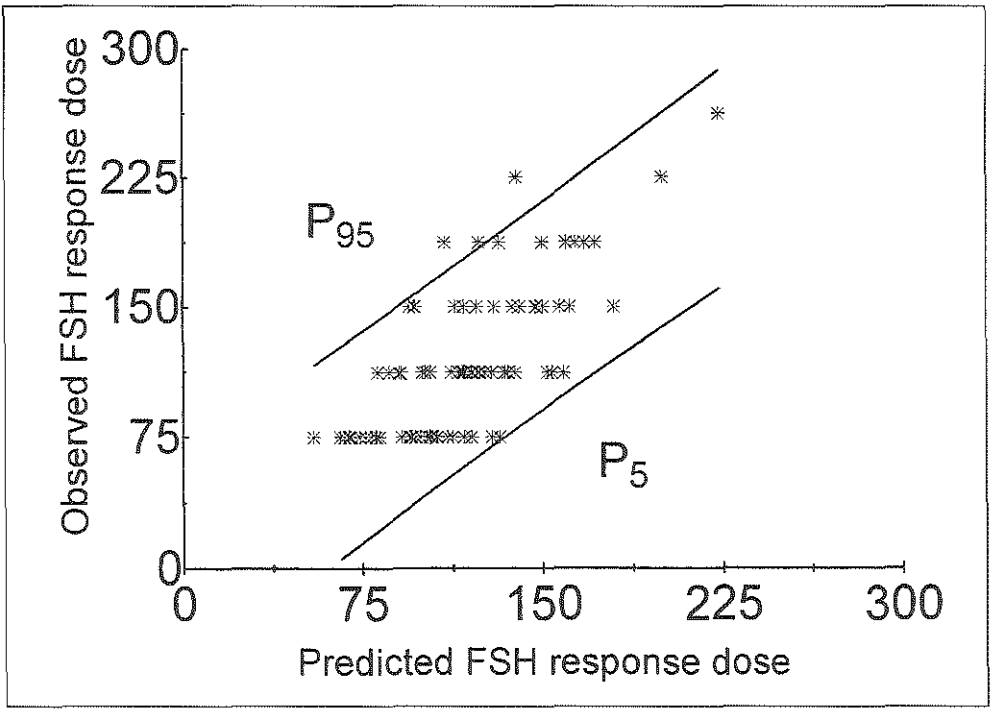

Figure 2 *Scatter plot of the observed and predicted FSH response dose by the model ([4 BMI (kg /m2)] + [32 CRA (yes = 1 or no = 0)] + [7 free IGF-I (ng/mL)] + [6 FSH (IU/L)] – 51) during ovulation induction by exogenous FSH using a low-dose step-up regimen in 90 normogonadotropic anovulatory infertile patients. P5 = the 5th percentile and P95 = the 95th percentile of predicted probability.*

3.2.4 Discussion

The present study was designed to evaluate whether initial clinical, endocrine and sonographic characteristics of normogonadotropic oligoamenorrheic infertile women who failed to ovulate or conceive after CC induction of ovulation could predict the amount of exogenous FSH required for ovarian response during a low-dose step-up regimen. The aim of this stimulation protocol is to apply the lowest possible daily dose of exogenous gonadotropins to gradually surpass the individual FSH threshold in order to achieve follicular maturation and subsequent ovulation. This concept was introduced by Brown (Brown *et al.*, 1978) and indicates that each woman has a given threshold requirement for FSH below which follicular development does not occur. After sufficient ovarian stimulation by exogenous FSH, the dominant follicle may be selected in these anovulatory patients (van Santbrink *et al.*, 1997a). Distinct differences in the individual FSH threshold level

may be held responsible for the relatively high incidence of complications such as multiple gestations or OHSS (Schoemaker *et al.*, 1993).

The FSH threshold may represent the severity of ovarian abnormalities in these patients. Up to 30% of normogonadotropic anovulatory women do not respond to increased endogenous FSH stimulation brought about by CC administration suggesting a high FSH threshold (Imani *et al.*, 1998; Imani *et al.*, 2000). These patients are characterized as obese, hyperandrogenic, amenorrheic women with an augmented ovarian volume (Imani *et al.*, 1998; Imani *et al.*, 2000).

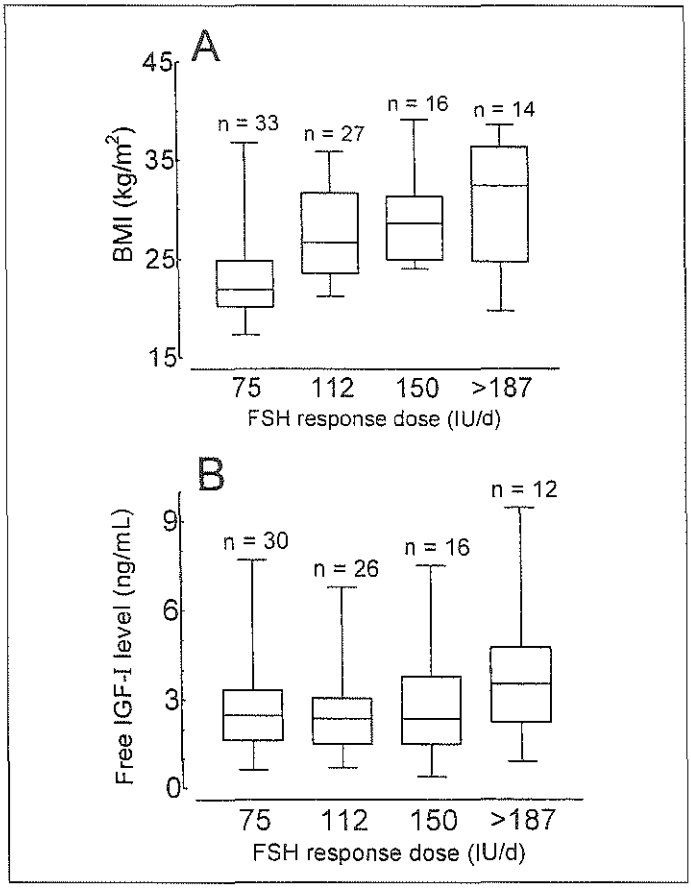

Figure 3 *Two initial screening parameters of 90 normogonadotropic anovulatory infertile patients separated in 4 different FSH response dose groups (75, 112.5, 150, or ≥ 187.5 IU/day), during ovulation induction by exogenous FSH using a low-dose step-up regimen. Data are presented as box and whisker plots: Boxes encompass values between the 25th and the 75th percentile, horizontal lines represent median values, and "whiskers" give the 95% range of values. The following initial prognostic factors have been depicted: BMI (A) and Free IGF-I (B). P values represent differences applying ANOVA (A: P < 0.001; B: P <0.004).*

The present study demonstrates that BMI and / or serum leptin levels, cycle history (oligomenorrhea *vs.* amenorrhea), ovarian response to CC medication (CCF *vs.* CRA), initial serum FSH, free IGF-I and IGFBP-1 levels were significantly correlated with the individual FSH response dose. In a multivariate analysis BMI, ovarian response to CC medication, initial serum free IGF-I and FSH level entered into the final model. The combination of these parameters showed good predictive probability within the final model with an R^2 of 0.54. BMI seems to be the most significant factor. The dose formula based on BMI alone is as follows: Predicted FSH response dose = 4.5 x BMI (kg/m^2) + 1.5 with a R^2 of 0.29 compared to 0.54. Predictive power lost is 0.25 if a single parameter (BMI) is used (data not shown).

Overweight amenorrheic patients require significantly higher FSH doses. BMI and initial serum leptin levels appear to be the most prominent predictors of the FSH response dose which is likely to be caused by an augmented ovarian FSH threshold in the patients. Differences in absorption and distribution of exogenous FSH in obese women may also be involved (Mannaerts *et al.*, 1993). A direct relationship between body weight and the amount of exogenous gonadotropins needed to induce ovulation in low-dose step-up cycles has previously been reported (Chong *et al.*, 1986; Hamilton-Fairley *et al.*, 1992). Previous reports from our group on prediction of ovarian response during CC induction of ovulation also stressed the importance of BMI and leptin (Imani *et al.*, 1998; Imani *et al.*, 2000). Although only BMI enters into the final model, leptin would enter in case information regarding BMI is not available (data not shown). This may be due to significant correlations between serum leptin levels and BMI, free IGF-I and CRA (Imani *et al.*, 2000).

Leptin, the hormone product of the obesity gene (Zhang *et al.*, 1994), inhibits IGF-I augmentation of FSH-stimulated estradiol production *in vitro* suggesting a direct action at the ovarian level (Agarwal *et al.*, 1999). However, the present findings are consistent with a limited direct role of leptin in the pathophysiology of PCOS. Serum leptin levels are positively correlated with fasting insulin levels in PCOS women and are not altered compared to normo-ovulatory women (Laughlin *et al.*, 1997). A positive influence of insulin on fasting leptin levels (independent of adiposity) has been reported in PCOS, suggesting that the opposing effects of hyperinsulinemia (stimulatory) and insulin resistance (inhibitory) may negate the impact of insulin excess and account for the maintenance of normal leptin levels (Laughlin *et al.*, 1997). In addition, weight reduction in obese women with PCOS may result in normalization of insulin resistance, androgen metabolism, and alleviation of ovarian abnormalities (Clark *et al.*, 1995). These observations underline the crucial role of body weight for ovarian function.

The present study demonstrates for the first time that the ovarian response to preceding CC medication predicts ovarian response during subsequent stimulation

by exogenous FSH. This finding confirms the concept previously proposed by our group indicating that CRA patients may suffer from a more serious ovarian abnormality which is more resistant to stimulation by FSH (Imani *et al.*, 1998; Imani *et al.*, 2000). Patients who ovulated after CC but failed to conceive exhibit a lower FSH threshold and may subsequently require lower dose of exogenous FSH to reach sufficient ovarian stimulation.

Both free IGF-I and IGFBP-1 levels were previously shown to be significantly different in PCOS patients compared with normo-ovulatory controls (van Dessel *et al.*, 1999). In the current study, free IGF-I predicts the FSH threshold level in anovulatory women independent from other assessed factors. Free IGF-I as well as fasting serum insulin levels are significantly higher in patients who have a higher FSH response dose, whereas serum IGFBP-1 is significantly lower. In fact, free IGF-I as well as insulin have been shown to directly augment theca cell androgen production *in vitro* (Legro *et al.*, 1998) and may therefore play important roles in perpetuating ovarian dysfunction in PCOS. This may also increase FSH requirements to induce follicle development. IGFBP-1 was also significantly lower in patients remaining anovulatory after CC, as shown previously (Imani *et al.*, 2000). Free IGF-I is not a commonly performed assay in daily clinical practice. However, the FSH response dose formula based on elimination of free IGF-I and substitution of insulin or insulin / glucose ratio did not improve R^2 of the model. Predicted FSH response dose = 3.5 x BMI (kg/m^2) + 35.6 x CRA + 6.7 x FSH + 2.6 x insulin / glucose ratio – 32.5 with a R^2 of 0.49 instead of R^2 of the prediction model (0.54) which uses free IGF-I. R^2 lost is 5 by using a model which uses serum insulin / glucose ratio instead of free IGF-I (data not shown).

In the present study, initial serum FSH levels predict the FSH response dose in anovulatory patients suggesting that baseline FSH levels are also associated with the FSH threshold in these women, despite the fact that concentrations are below the threshold. This is in line with previous observations in normo-ovulatory women where maximum early follicular phase FSH concentrations also represent the FSH threshold (Schipper *et al.*, 1998).

Inhibin B is produced by healthy antral follicles in the early follicular phase of normal menstrual cycle (Groome *et al.*, 1996). Therefore inhibin B may represent the number of healthy follicles in polycystic ovaries and may serve as a marker for the severity of ovarian dysfunction in anovulatory patients. However, this contention is not supported by the current observations since inhibin B does not predict the FSH threshold.

Amenorrheic or severely oligomenorrheic patients exhibit a higher FSH response dose. This finding is in agreement with previous observations regarding

ovarian response after CC (Imani *et al.*, 1998) suggesting that the cycle abnormality may reflect the severity of ovarian dysfunction in anovulatory patients. This easy to collect information seems to be a major prognostic factor in prediction of ovarian response to FSH stimulation by either CC or exogenous FSH.

To our knowledge this is the first time a model has been developed to predict the individual FSH response dose in anovulatory patients on the basis of initial screening characteristics. By choosing the appropriate starting dose chances of OHSS or multiple gestations may diminish. However, this contention should be confirmed in subsequent prospective trials. Obese, amenorrheic, CRA women exhibit a higher FSH response dose. The starting dose of exogenous FSH should be higher in these women in order to reach sufficient ovarian stimulation. Monitoring of ovarian response for an extended period of time may be prevented in patients receiving FSH doses below the threshold. This approach may increase patient convenience and render treatment more efficient. In contrast, non-obese, oligomenorrheic, CCF women exhibit significantly lower FSH response dose. The great majority of these women may reach sufficient stimulation by a fixed daily dose of 50-75 IU/day. Adjusting the initial FSH dose, particularly in step-down regimens, may render this treatment modality safer in women with a low FSH threshold. The step-down regimen has initially been developed with a fixed starting dose of 150 IU/d (van Santbrink *et al.*, 1995).

Although this initial dose is adequate for the majority of patients, stimulation should be cancelled in some cases due to hyperresponse. Currently, this represents the major drawback of the step-down approach (Fauser *et al.*, 1997). It would be a distinct step forward in the further development of this decremental regimen in case patient characteristics could identify women in advance who would benefit from a lower starting dose of FSH.

In conclusion, it is possible to predict the individual FSH response dose in anovulatory infertile women using BMI, ovarian response during preceding CC medication, and serum free IGF-I and FSH concentrations. The possibility to predict the individual FSH threshold may increase safety, efficiency and patient convenience of low-dose regimens for gonadotropin induction of ovulation by the proper assessment of the appropriate starting dose for a given patient. This may represent a distinct step forward. Time-consuming low dose increments may be overcome by starting with a high dose in women with an augmented FSH threshold. In contrast, starting with an individualized dose in step-down regimens may reduce chances for hyperresponse in women with a low FSH threshold. These approaches should be further evaluated in prospectively designed comparative trials.

3.3 Prediction of Chances for success or Complications in Gonadotropin Ovulation Induction Using a Step-down Protocol in Anovulatory Infertility

3.3.1 Introduction

Exogenous gonadotropin preparations have extensively been used since their introduction into clinical practice in 1958. Next to ovarian hyperstimulation for assisted reproduction these preparations have been used for induction of ovulation in anovulatory infertile patients who failed to ovulate or conceive during preceding anti-oestrogen medication (Schwartz *et al.*, 1981; Lunenfeld *et al.*, 1985; Insler *et al.*, 1988; Kelly *et al.*, 1990; Fauser *et al.*, 1993c; Franks *et al.*, 1994; Fauser *et al.*, 1997). Exogenous gonadotropins elicit increased ovulation rates (up to 90%) compared to clomiphene citrate (CC) with comparable cumulative conception rates (65%) (van Santbrink *et al.*, 1995; White *et al.*, 1996; Imani *et al.*, 1998; Imani *et al.*, 1999). However, this treatment modality is associated with higher chances for complications such as ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies (Fauser *et al.*, 1997).

Distinct individual differences in the amount of FSH required to elicit an ovarian response (referred to as the "FSH threshold") has been proposed to be the main factor in hyperresponse and severe complications in some patients (Brown *et al.*, 1978). Various low-dose gonadotropin protocols have been introduced recently in order to reduce the chances for complications. The low-dose step-up regimen aims at slowly and prudently surpassing the FSH threshold (White *et al.*, 1996), whereas the low-dose step-down protocol (van Santbrink *et al.*, 1995; van Santbrink *et al.*, 1997a) aims to mimic the physiological conditions during the luteo-follicular transition of spontaneous ovulatory cycles (van Santbrink *et al.*, 1995).

We previously designed a prospective longitudinal follow-up study attempting to develop model predicting individual outcome of ovulation induction using pretreatment clinical, endocrine and sonographic screening characteristics in normogonadotrophic anovulatory women. Analyses was performed separately for CC (Imani *et al.*, 1998; Imani *et al.*, 1999; Imani *et al.*, 2000) and FSH (Imani *et al.*, 2001a). An equation has been introduced to predict the individual FSH response dose for gonadotropin induction of ovulation (Imani *et al.*, 2001a). Body weight, ovarian response to CC, and cycle history (amenorrhea) were the major factors predicting augmented FSH threshold. It would be of distinct clinical significance in case initial screening characteristics could also predict clinical outcome of gonadotropin ovulation induction (*i.e.* chances for success or complications). Some previous studies suggested that the occurrence of polycystic ovaries (White *et al.*,

1996), overweight (Hamilton-Fairley *et al.*, 1992) and duration of stimulation / total amount of exogenous FSH (van Santbrink *et al.*, 1997a) may be important factors during ovarian stimulation applying exogenous FSH (Fauser *et al.*, 1997). The present prospective follow-up study was designed to investigate whether pre-treatment clinical, endocrine, and sonographic screening characteristics of normogonadotrophic anovulatory women, predict individual chances for complications (*i.e.* multi-follicular growth) or success (conception) prior to initiation of this treatment strategy.

3.3.2 Materials and Methods

Subjects

One hundred and fifty two women attending our infertility unit March 1993 and December 1999 were included in the present study using the following criteria: a) oligomenorrhea (bleeding intervals between 35 days and 6 months) or amenorrhea (bleeding interval > 6 months), b) serum FSH levels within normal limits (1-10 IU/L) (van Santbrink *et al.*, 1995; Schipper *et al.*, 1998) and normal serum prolactin and thyroid-stimulating hormone levels, c) spontaneous menses or positive bleeding response to progestagen withdrawal, d) being previously treated unsuccessfully in our fertility unit with CC medication (failure to ovulated, or failure to conceive in ovulatory CC-cycles) (Imani *et al.*, 1998; Imani *et al.*, 1999; Imani *et al.*, 2000), e) body mass index (BMI) (weight divided by square height) > 18 kg/m^2, f) between 19-40 years of age, g) a total motile sperm count (TMC = ejaculate volume (ml) x sperm concentration (10^6/ml x percentage of progressive motile sperm) of the partner above 1 million, i) negative history for any tubal pathology plus negative chlamydia antibody titer, or negative hysterosalpingography or laparoscopic inspection, and ultimately j) no indication for intra-uterine insemination. Study approval was obtained from the human subjects committee of the Erasmus Medical Center and informed consent was obtained from all subjects.

Standardised initial clinical, sonographic, and endocrine screening took place prior to initiation of exogenous gonadotropins using a low-dose step-up regimen, as described previously (Imani *et al.*, 2000). Clinical screening included age, infertility duration and history (primary *vs.* secondary), cycle history, ovarian response to previous CC medication, BMI, waist-to-hip ratio (WHR), any other previous medication and/or surgery. Transvaginal sonographic (TVS) screening included assessment of the ovarian stroma echogenicity (arbitrarily classified from 1 to 3 per ovary), ovarian volume (ml) and total number of follicles (both ovaries), as described previously (Pache *et al.*, 1991; Pache *et al.*, 1992; van Santbrink *et al.*, 1997b).

Sonographic monitoring was performed by a single observer (B.I.), using an ultrasound machine (model EUB-415, Hitachi Medical Corp., Tokyo, Japan) with a 6.5-MHZ transvaginal transducer.

Hormone assays

Endocrine screening included serum assays for FSH, prolactin, thyroid-stimulating hormone, luteinising hormone (LH), oestradiol (E_2), androstenedione (AD), testosterone (T), and sex-hormone binding globulin (SHBG), as described previously (Imani *et al.*, 1998; Imani *et al.*, 1999). In addition, serum was also assayed for fasting insulin and glucose, free and total IGF-I, IGFBP-1, IGFBP-3 and leptin concentrations as described previously (Imani *et al.*, 2000). The method of blood withdrawal, the assays used and the intra- and inter assay coefficients of variation valid for this study have all been previously described (Imani *et al.*, 1998; Imani *et al.*, 1999; Imani *et al.*, 2000).

Study protocol

The treatment protocol and assessment of ovarian response were as follows. Exogenous FSH was initiated on day 3-5 after a spontaneous or progestagen-induced withdrawal bleeding. A low dose step-up regimen for induction of ovulation using either daily intra-muscular (IM) injections of urinary FSH (Metrodin HP®, Serono, Serono, Switzerland) or sub-cutaneous (SC) injections of recombinant FSH (Gonal-F®, Serono) was used for the stimulation of follicle growth and assessment of the individual FSH response dose (Imani *et al.*, 2001a). Monitoring of dominant follicle development (i.e sonographic visualisation of a follicle ≥ 10 mm (Pache *et al.*, 1990; van Santbrink *et al.*, 1995) was performed every 2 or 3 days until human chorionic gonadotropin (hCG) (Pregnyl®, Organon, Oss, The Netherlands) was administered. A single I.M. dose of 5,000 IU hCG was administered on the day upon which one or two follicles ≥ 18 mm could be visualised. In case three or more follicles ≥ 16 mm were present, stimulation was cancelled. Ovulation after hCG administration was determined by the sonographic signs of the collapse of the dominant follicle and mid-luteal progesterone (P) levels above 25 nmol/l.

Low-dose, step-up protocol

The starting dose of FSH was one ampoule (75 IU) per day. The first increase in FSH dose by half an ampoule (37,5 IU) per day was based on the absence of sonographic visualisation of a follicle ≥ 10 mm after 7 days. The FSH dose was increased subsequently by half an ampoule (37,5 IU) per day for the following 7 days in case an ovarian response was still lacking. In case of sufficient ovarian response, exogenous FSH dose was unaltered until administration of hCG. No

luteal support was provided. The effective FSH response dose in some patients was 75 IU/day. This dose of FSH was sustained until the day hCG could be administered.

Step-down protocol

The starting dose of FSH was chosen on the basis of one of the two following criteria: 1) Three categories of body mass index (18-22, 23-28, >29) have been used based on initial experience of our group before 1996. We started directly with step-down protocol using either $1\,^1/_2$, 2, or $2\,^1/_2$ ampoules per day (n=58 patients). The first decrease in dose by half an ampoule (37,5 IU) per day was based on visualisation of a follicle growth ≥ 10 mm in diameter as described previously (van Santbrink *et al.*, 1997a). A further dose decrease (each time by 37,5 IU/day) was performed every 3 days in case follicular growth continued to a minimum dose of one ampoule (75 IU) per day until the day hCG could be administered. The initial dose was increased by half an ampoule (37,5 IU) per day in case ovarian response remained absent after 5 days. If follicular growth remained absent over the following 10 days (2 incremental steps of 37,5 IU/day), further medication was withheld and the cycle was cancelled. No luteal support was provided. 2) From 1996 an initial low-dose step-up cycle have been applied in our clinic to evaluate individual FSH response dose (n=94 patients). Arbitrarily we used a dose of half an ampoule (37,5 IU) per day of FSH above the response dose to start the subsequent step-down cycle.

Fixed dose 1 ampoule/day protocol

The effective FSH response dose in this group of patients during the low-dose step-up induction cycle was 1 ampoule/day. Thus, the starting dose of FSH was 1 ampoules (75 IU) and kept constant till the day of sonographic visualisation of a follicle ≥ 10 mm. This dose was sustained until the day hCG could be administered. The initial dose was increased by a $^1/_2$ ampoule/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 $^1/_2$ ampoule/day), further medication was withheld and the cycle was cancelled. No luteal support was provided.

Data analysis

We used univariate and multivariate logistic regression analyses for comparison of patients who developed multiple large dominant follicles (more than 1 follicle > 15 mm) (van Santbrink *et al.*, 1997a). The multivariate odds ratio is provided for the factors significant in the multivariate analyses. For instance multivariate odds ratio (95% CI) of serum AD = 1.05 (1.02-1.08) (Table 3). This means a 1-unit increase in a given patient's serum AD level gives her a 5% higher probability of develop-

ing multifollicular growth after exogenous gonadotropin induction of ovulation.

Univariate and multivariate Cox regression was used for life table analysis of conception rates during exogenous gonadotropin induction of ovulation. The number of gonadotropin induced cycles was the time variable for multivariate analyses. Censoring was defined as definitive discontinuation of gonadotropin therapy without conception or end of follow-up. The Log Rank test has been used to denote statistical significance in life table analyses. A P-value of 0.05 was chosen as threshold level for statistical significance. The multivariate analysis was performed with the method of forward stepwise selection to gain a better insight in the interdependence between initial screening parameters. The prognostic impact of variables was expressed as a fecundability ratio, which is equivalent to the hazard ratio in survival analysis. Data were analysed using the commercially available software package SPSS (Chicago, IL, USA).

3.3.3 Results

A total number of one hundred and fifty two patients fulfilled the in/exclusion criteria and were treated with exogenous gonadotropin induction of ovulation. Initial clinical, endocrine, and sonographic screening characteristics of the overall group of anovulatory infertile patients, the group with mono or multi-follicular development, and those who did or did not conceive are depicted in Tables 1 and 2. The results of univariate analyses for comparison between these groups of patients (women who did or did not reach a monofollicular development and those who did or did not conceive) have been also depicted in Table 1 and Table 2. In 198 (45%) cycles multi-follicular growth occurred and in 50 (11%) cycles no human chorionic gonadotropin (hCG) was given and the stimulated cycle was cancelled due to multi-follicular growth and potential risks for multiple pregnancy or ovarian hyperstimulation syndrome (OHSS). Ovarian response to the preceding CC treatment, initial serum LH, FSH, T, and mean number of follicles were significantly different for patients who developed mono vs. multi-follicles during ovarian stimulation applying step-down dose regimen. The results of the forward step-wise multivariate logistic regression analyses of the patients who did or did not develop a single dominant follicle are depicted in Table 3.

Seventy-nine women (52%) conceived. Ten miscarried (13%) and the remaining ongoing pregnancies ended in 67 singleton, one twin, and one triplet pregnancy. The life table analysis of CCR of the overall group of patients is depicted in Figure 1. A cumulative conception rate (CCR) of 60% was reached within 6 induced cycles (Figure 1). In univariate Cox regression analyses, patients age, initial serum free IGF-I and IGF-I were significantly different between patients who did or did not

conceive following gonadotropin induction of ovulation (Table 2). Multivariate Cox regression analyses of screening parameters for prediction of conception following gonadotropin induction of ovulation has been depicted in Table 4. Woman's age, initial serum IGF-I and T entered the final model (AUC = 0.69). Age (cut-off of 30 yr), ovarian response during preceding CC medication (CC-responders vs. non-responders), and initial serum LH level (cut-off level of 0.7 IU/L) in univariate analyses for CCR are depicted in Figure 1.

The impact of predicted FSH response dose on treatment outcome (the number of dominant follicle maturation and conception) has been depicted in Figure 2. The impact of initial screening characteristics as a prognostic factor in 152 normogonadotropic anovulatory infertile patients on prediction of a single dominant follicle maturation has been depicted in Figure 3.

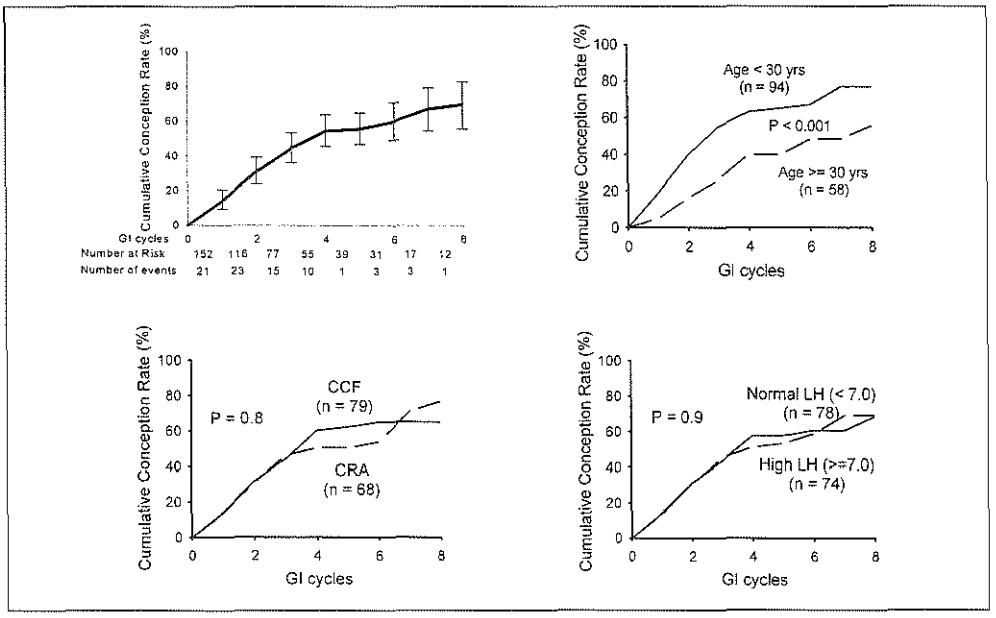

Figure 1 *Life table analysis of cumulative conception rates (CCR) in 152 normogonadotropic oligoamenorrheic infertile patients who failed to ovulate or conceive following preceding CC medication and were treated with ovulation induction by exogenous FSH applying a step-down dose regimen. CCR's (including absolute number of patients at risk and number of events, conceptions) are presented for the total study group (upper left panel) (vertical lines represents 95% Confidence Intervals). Univariate analysis of cumulative conception rates in the same patients. Initial screening parameters which have been depicted are as follows: 1) Patient's age (cut-off level at 30 years) (upper right panel). 2) Ovarian response to preceding CC medication (CRA vs. CCF) (lower left panel). 3) Initial serum LH concentrations (cut-off level of 7.0 IU/L) (lower right panel). n represents the initial number of patients at risk. P = Log Rank test P value.*

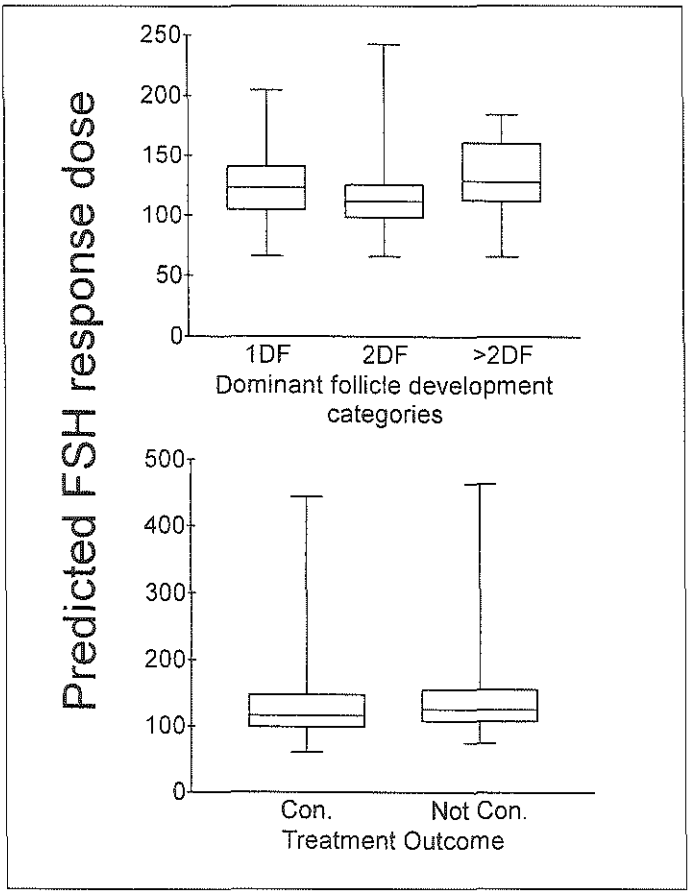

Figure 2 *Predicted FSH response dose as a prognostic factor in 152 normogonadotropic anovulatory infertile patients who failed to conceive following preceding CC medication and were treated with ovulation induction by exogenous FSH applying a step-down dose regimen. Data are presented as box and whisker plots: Boxes encompass values between the 25th and the 75th percentile, horizontal lines represent median values, and "whiskers" give the 95% range of values. Two treatment outcome have been depicted: Dominant follicle development in three categories (A) and conception (B). P > 0.05 applying ANOVA.*

3.3.4 Discussion

The present study was designed to evaluate whether initial clinical, endocrine and sonographic characteristics of 152 clomiphene resistant or failure anovulatory infertile women may predict the chance of treatment outcome during gonadotropin induction of ovulation applying a step-down dose regimen. Although gonadotropin ovulation induction have been the focus of vast number of investigators

during the last four decades, a prospective follow-up study to predict the chance of ongoing pregnancies during this treatment strategy is lacking. Our group, however, focused on a prospective follow-up study in entire WHO 2 anovulatory infertile women since 1993. Several prediction models have been developed. These models may be used to predict CC ovulation induction outcome (ovulation, ongoing pregnancy, or live birth) as well as the amount of the exogenous gonadotropin required to reach an ovarian response in women who failed to ovulate or conceive following CC (Imani *et al.*, 1998; Imani *et al.*, 1999; Imani *et al.*, 2000; Imani *et al.*, 2001a; Imani *et al.*, 2001b). In brief, obese amenorrheic hyperandrogenic women are more likely to remain anovulatory following CC. However, from those women who reach a sufficient ovarian response, young amenorrheic women are more likely to conceive leading to a live birth after CC (Imani *et al.*, 1998; Imani *et al.*, 2001b). It has been recently reported that the amount of exogenous FSH necessary for a sufficient ovarian response during exogenous gonadotropins can be predicted in women who failed to ovulate or conceive following CC applying four initial screening parameters of the following equation: The individual FSH response dose (IU/d) = (4 A + 32 B + 7 C + 6 D) − 51 in which A = body mass index (BMI; kg /m^2), B = clomiphene resistant anovulation CRA (yes = 1 or no = 0), C = initial free insulin-like growth factor-I (free IGF-I; ng/mL), and D = initial serum FSH (IU/L) (Imani *et al.*, 2001a).

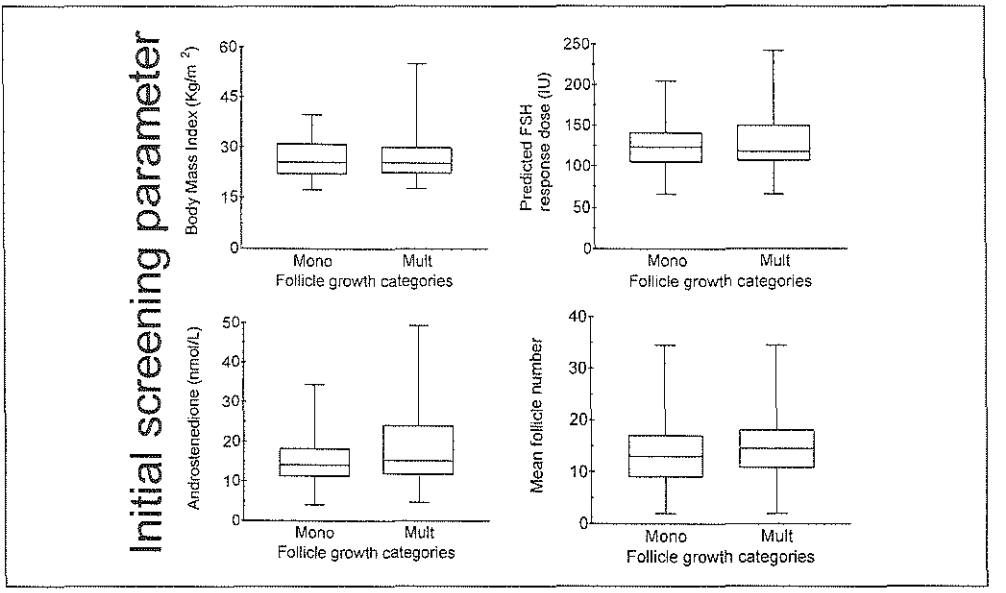

Figure 3 *The impact of body mass index, predicted FSH response dose, serum androstenedione, and mean follicle number on follicle development following gonadotropin ovulation induction applying step-down regimen in 152 normogonadotropic oligoamenorrheic infertile patients who failed to ovulate or conceive following preceding CC.*

TABLE 1. Initial clinical, endocrine, and ultrasound screening characteristics (mean ± SD) of 152 normogonadotropic anovulatory infertile women, separately for cycles with mono (n = 244) or multi-follicular (n = 198) growth after FSH induction of ovulation using a step-down dose regimen.

	Overall group (n patient = 152)	Mono-follicular growth (n cycle = 244) [a]	Multi-follicular growth (n cycle = 198) [a]	P [b]	AUC [b]
Screening parameters					
Clinical					
Age (yrs)	29 ± 4	29 ± 4	29 ± 4	NS	-
Amenorrhea (n)	52	68 (24%)*	62 (32%)*	NS	-
BMI (kg/m^2)	26.9 ± 6.0	26.8 ± 5.7	26.4 ± 5.7	NS	-
Waist-to-hip ratio (WHR)	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	NS	-
CC resistant anovulation (n)	68 (100%)	102 (43%)*	106 (53%)*	0.02	0.56
Predicted FSH response dose (IU)	120 ± 33	120 ± 33	120 ± 33	NS	-
Endocrine					
LH (IU/L)	8.0 ± 4.6	7.9 ± 4.6	8.8 ± 4.2	0.03	0.58
FSH (IU/L)	4.9 ± 1.5	5.1 ± 1.6	4.8 ± 1.3	0.04	0.55
E_2 (pmol/L)	243 ± 124	254 ± 151	267 ± 135	NS	-
AD (nmol/L)	15.6 ± 7.8	15.2 ± 6.3	18.1 ± 9.1	< 0.001	0.59
T (nmol/L)	2.6 ± 1.1	2.6 ± 1.0	2.9 ± 1.2	< 0.001	0.60
SHBG (nmol/L)	48.8 ± 27.3	51.1 ± 26.8	50.6 ± 24.5	NS	-
FAI (T x 100 / SHBG)	7.4 ± 6.1	7.0 ± 5.5	7.6 ± 5.6	NS	-
Insulin (mU/L)	14.4 ± 10.2	14.1 ± 10.2	15.0 ± 11.1	NS	-

Insulin/glucose ratio	3.4 ± 2.3	3.4 ± 2.3	3.7 ± 2.4	NS	-
Inhibin B (ng/L)	159 ± 117	164 ± 123	182 ± 127	NS	-
IGF-I (ng/mL)	231 ± 82	220 ± 84	214 ± 78	NS	-
Free IGF-I (ng/mL)	2.7 ± 1.7	2.5 ± 1.6	2.7 ± 1.5	NS	-
IGFBP-1 (ng/mL)	24.5 ± 18.1	24.0 ± 18	25.4 ± 17	NS	-
IGFBP-3 (ng/mL)	3,406 ± 749	3,359 ± 773	3,295 ± 593	NS	-
Leptin (ng/mL)	20.5 ± 16	20.8 ± 16	20.6 ± 15	NS	-
Ultrasound					
Total stroma score[c]	3.8 ± 1.2	3.8 ± 1.2	3.7 ± 1.2	NS	-
Mean ovarian volume (mL)	10.8 ± 4.6	10.8 ± 4.6	10.2 ± 4.6	NS	-
Mean follicle number	14 ± 6	14 ± 6	14 ± 6	0.07	0.56

[a] Cycles with poor response (n=29 cycles; absent follicle growth beyond 14 mm) were excluded.

[b] *P* value of univariate logistic regression analyses, and area under the ROC curve.

[c] Arbitrarily defined as one to three per ovary (both ovaries added).

[d] Polycystic ovaries defined as mean ovarian volume ≥ 10.8 mL and/or mean follicle number per ovary ≥ 10 (van Stanbrink et al, *Fertil Steril* 1997)

* Prevalence (%) of characteristics with the specific group of women.

TABLE 2. Initial clinical, endocrine, and ultrasound screening characteristics (mean ± SD) and sperm parameters (median and range) of 152 clomiphene resistant or failure anovulatory infertile women, separated for patients who did or did not conceive after exogenous gonadotropin induction of ovulation using a step down regimen.

	Conceived (n = 79; 52%)	Did not conceive (n = 73; 48%)	P [a]	AUC [a]
Screening parameters				
Clinical				
Age (yrs) [b] *	29 ± 4	28 ± 4	0.02	0.61
Infertility duration (yrs)	2.7 ± 1.7	3.2 ± 2.2	NS	-
Primary infertility (n)	57 (53%)	50 (47%)	NS	-
Amenorrhea (n)	28 (54%)	24 (46%)	NS	-
BMI (kg/m^2)	26.4 ± 6.2	27.5 ± 5.8	NS	-
Waist-to-hip ratio (WHR)	0.8 ± 0.1	0.8 ± 0.1	NS	-
CRA (n)	35 (51%)	33 (49%)	NS	-
Predicted FSH response dose (IU)	119 ± 33	121 ± 33	NS	-
Endocrine				
LH (IU/L)	8.4 ± 5.0	7.7 ± 4.2	NS	-
FSH (IU/L)	4.9 ± 1.5	5.0 ± 1.5	NS	-
E_2 (pmol/L)	232 ± 125	256 ± 122	NS	-
AD (nmol/L)	15.7 ± 8.5	15.4 ± 7.0	NS	-
T (nmol/L)	2.5 ± 1.1	2.6 ± 1.1	0.09	0.58
SHBG (nmol/L)	46.9 ± 26.9	50.9 ± 27.8	NS	-
FAI (T x 100 / SHBG)	7.4 ± 5.9	7.5 ± 6.3	NS	-

Insulin (mU/L)	15.1 ± 11.4	13.8 ± 8.9	NS	-
Insulin/glucose ratio	3.5 ± 2.5	3.2 ± 2.1	NS	-
Inhibin B (ng/L)	156 ± 101	163 ± 133	NS	-
IGF-I (ng/mL)	251 ± 81	209 ± 78	0.006	0.61
Free IGF-I (ng/mL)	3.0 ± 1.7	2.4 ± 1.7	0.04	0.061
IGFBP-1 (ng/mL)	24.5 ± 18.6	24.5 ± 17.7	NS	-
IGFBP-3 (ng/mL)	3477 ± 645	3327 ± 848	0.08	0.56
Leptin (ng/mL)	20.1 ± 15	20.9 ± 17	NS	-
Ultrasound				
Total stroma score[c]	3.7 ± 1.1	3.9 ± 1.3	NS	-
Mean ovarian volume (mL)	10.5 ± 4.1	11.2 ± 5.1	NS	-
Mean follicle number	14 ± 6	14 ± 7	NS	-
Total motile sperm count (TMC)	67 (1-661)	39 (1-428)	NS	-
% Normal morphology	25 (8-90)	23 (1-48)	NS	-

[a] *P* value of univariate logistic regression analyses and area under the ROC curve of initial parameters for comparison of cycles with mono or multifollicualr growth following exogenous gonadotropin induction of ovulation using a step down regimen

[b] * corresponds to *P* value of univariate Cox regression analyses for comparison of patients who did or did not conceive following exogenous gonadotropin induction of ovulation (* = $P < 0.05$, ** = $P < 0.01$).

[c] Arbitrarily defined as one to three per ovary (both ovaries added).

In the present study, the predicted FSH response did not predict treatment outcome during gonadotropin induction of ovulation applying step-down dose regimen *i.e* multi-follicular development, cycle cancellation or ongoing pregnancy (Table 1 and Figure 2). The major factor involved in prediction of exogenous FSH requirement for ovarian response - obesity - is not a predictor for multi-follicular growth. Factors associated with obesity such as BMI, WHR or serum leptin level do not predict treatment outcome following exogenous FSH induction of ovulation applying step-down regimen. Previous reports from our group indicated that obesity and cycle history (amenorrhea *vs.* oligomenorrhea) are strong predictors of patients remaining anovulatory following CC (Imani *et al.*, 1998). These factors also predict patient's exogenous FSH requirement for initiation of an ongoing follicle growth in FSH-induced cycles applying a low-dose step-up regimen (Imani *et al.*, 2001a). Cycle history (amenorrhea *vs.* oligomenorrhea) had no impact on exogenous FSH treatment outcome in the present study. The remaining factors of the model for prediction of FSH response dose, such as CRA or serum free IGF-I level, predict exogenous gonadotropin induction of ovulation outcome. CRA with a low initial serum FSH level exhibit more chance to develop a multi-follicular growth compared to CCF patients with a normal FSH serum concentration. Serum free IGF-I - which entered in multivariate model for prediction of FSH response dose - predicts patients who have higher chance to conceive during gonadotropin ovulation induction in univariate analyses. These findings may indicate that impact of an augmented FSH threshold does not necessarily indicate higher chance of multi-follicular growth or conception of the cycle during step-down cycles. This may be due to serum endogenous FSH accumulation pattern differences between low-dose step-up vs. step-down regimen (van Santbrink *et al.*, 1997a).

Initial serum AD and mean follicle number entered into the model predicting the chance of multiple follicle development during gonadotropin induction of ovulation. Hyperandrogenic patients who present with an increased number of unstimulated follicles during pelvic ultrasound screening are more likely to develop multiple follicle growth and consequently cancellation of the cycle. In univariate analyses, initial serum LH level which predict CC medication outcome, is a predictor for multiple follicle development during exogenous gonadotropin induced cycles applying step-down dose regimen. CRA patients, however, exhibit a high chance of multi-follicular development compared to CCF during exogenous FSH induction of ovulation. Patients with low initial serum FSH level tend to develop multiple follicle growth. We used our strict criteria for definition of PCO and/or hyperandrogenemia in our study. Both these factors predict multiple follicle growth during exogenous gonadotropin ovulation induction applying step-down protocol.

Initial serum LH, LH/FSH ratio, AD, and T levels are significantly higher and, in contrast, serum FSH level is lower in patients who exhibit higher chance of multiple follicle development during gonadotropin ovulation induction applying step-down regimen. Patients who remain anovulatory following CC, women who present with increased number of follicles during assessment of initial pelvic ultrasound screening prior to ovarian stimulation, and patients defined as exhibiting PCO are at high risk to develop multiple follicle development during exogenous FSH induction of ovulation. When a multivariate analysis for prediction of multiple follicle development chances is applied, initial serum AD and mean follicle number are entered into the model (see Table 3). The impact of initial serum AD level is the highest and the area under the receiver operating characteristics of the present model is 0.60. Serum SHBG or FAI level does not predict patients who are at high risk for multiple follicle development during ovarian stimulation applying exogenous gonadotropin. However, predictive power of serum FAI is the highest in our report on prediction of CRA patients who suffer from severe ovarian dysfunction (Imani *et al.*, 1998; Imani *et al.*, 2000). The impact of FAI is due to significance of both serum SHBG and T in prediction of patients remaining anovulatory following CC. Predictors of ovarian response between these two ovulation induction strategies differs presumably due to differences between mechanism and mode of action of CC compared to exogenous FSH.

Initial serum insulin, insulin/glucose ratio, or inhibin B level had no predictive value for prediction of chances for multiple follicle development during exogenous gonadotropin induction of ovulation. We have not been able to confirm that elevated level of these endocrine parameters may represent the magnitude of ovarian hypersensitivity for multiple follicle development during exogenous administration gonadotropin for ovulation induction applying a decremental dose regimen.

In the present study, cumulative conception rate (CCR) of %65 have been reached within eight consecutive gonadotropin ovulation induction cycles. This is in agreement with our previous report on CCR in CC-induced ovulatory cycles (Imani *et al.*, 1999) and is similar to reports on spontaneous conception chances in normoovulatory women (Tietze *et al.*, 1968). This is also comparable to conception rates reported in the literature regarding exogenous gonadotropin induction of ovulation applying a low-dose step-up regimen (Hamilton-Fairley *et al.*, 1991; van Santbrink *et al.*, 1995) or a step-down protocol (van Santbrink *et al.*, 1995). Applying an initial dose-finding low-dose step-up cycle prior to step-down cycles, the starting dose for the first step-down cycle has been individualised and adjusted which has led to overall singleton pregnancies in women whom this approach has been applied. Moreover, strict cancellation criteria were used in patients with multiple follicle development or an imminent chance for multiple pregnancy.

Applying the present modified step-down dose regimen one may expect overall singleton pregnancy chances with no decrease in CCR.

In the present study initial serum IGF-I, age, and serum free IGF-I were significantly different comparing patients who conceived *vs.* those who did not conceive during exogenous gonadotropin induction of ovulation applying decremental dose regimen. Multivariate analyses revealed a final model including initial serum IGF-I, age, and serum T. The area under the receiver operating characteristics curve of the present model reached 0.69 (data not shown), which is comparable with that in the previous model predicting conception in CC-induced ovulatory cycles (0.68) (Imani *et al.*, 1998). The predictive power of initial serum IGF-I was the highest. Age entered in the second step. However, the predictive power of age in the present model is comparable with the impact of age in the model reported by our group for prediction of ongoing pregnancy following ovulatory CC-induced cycles. One year increase in patient's age reduces her chance for ongoing pregnancy of approximately 9 percent (footnote Table 4). To our knowledge, this is the first time a model is introduced for prediction of chances for ongoing pregnancy during gonadotropin induction of ovulation. The impact of age as the major factor for prediction of chances for conception following spontaneous, FSH-induced or CC-induced cycles has previously been reported. It should be taken into account that a significant number of young amenorrheic patients have reached live birth during the preceding CC-induced ovulatory cycles (Imani *et al.*, 2001b). This may indicate that predictive power of age would have been higher in case step-down regimen was applied as the first line treatment modality instead of CC. The predictive power of serum free IGF-I for prediction of ongoing pregnancy was significantly reduced when serum IGF-I entered into the model in the first step due to significant correlation between these two parameters.

The present study demonstrates for the first time that patients who remain anovulatory following CC have comparable chance for conception during gonadotropin induction of ovulation compared to women who failed to conceive despite the preceding 6 to 8 consecutive CC-induced ovulatory cycles. CRA exhibit no chance of conception due to persistent anovulation whereas CCF ovulate but do not conceive presumably due to impaired endometrial receptivity following consecutive ovulatory CC-induced cycles. During gonadotropin induction of ovulation, however, CCF benefit from comparable chance for ongoing pregnancy compared to CRA. Endometrial receptivity of women who respond to CC might be impaired due to its effect after long term exposure. However, this reduced fecundability rate may be enhanced once exogenous FSH preparations are applied.

TABLE 3. Forward stepwise multivariate analyses of initial screening characteristics for the prediction of developing multifollicular growth after FSH induction of ovulation using a step down regimen in 152 normogonadotropic anovulatory infertile patients (425 cycles).

Analyses steps	Univariate [a]	Multivariate	
	0	1	2 [b]
Screening parameters			
Clinical			
CRA	0.02	0.12	0.22
Endocrine			
LH (IU/L)	0.03	0.24	0.46
FSH (IU/L)	0.04	0.22	0.32
AD (nmol/L)	< 0.001	In model	In model [c]
T (nmol/L)	< 0.001	0.26	0.67
Ultrasound			
Mean follicle number	0.07	0.05	In model [d]

[a] Univariate logistic regression < 0.10

[b] Final model, Area under the ROC curve = 0.6

[c] Multivariate odds ratio (95% CI) = 1.05 (1.02-1.08).

[d] Multivariate odds ratio (95% CI) = 1.03 (1.00-1.07).

TABLE 4. Forward stepwise multivariate analyses of screening parameters for prediction of conception following exogenous gonadotropin induction of ovulation using a step down regimen in 152 clomiphene resistant or failure anovulatory infertile patients.

Analyses steps	Univariate [a]	Multivariate		
	0	1	2	3 [b]
Screening parameters				
Clinical				
Age (yrs)	0.02	0.02	In model	In model
Infertility duration (yrs)	0.8	0.8	0.7	0.8
Primary infertility (n)	0.9	0.9	0.8	0.7
CRA	0.6	0.8	0.2	0.8
Endocrine				
LH (IU/L)	0.8	0.7	0.9	0.2
T (nmol/L)	0.09	0.08	0.03	In model
IGF-I (ng/mL)	0.006	In model	In model	In model
Free IGF-I (ng/mL)	0.04	0.13	0.3	0.2

[a] Univariate Cox regression < 0.10

[b] Final model, multivariate fecundability ratio (95% CI) of IGF-I = 1.004 (1.001-1.006), of age = 0.91 (0.85-0.97), and of T = 0.74 (0.57-0.97). This mean that a 1 year increase in a given patient's age reduces her monthly probability of conception following exogenous gonadotropin induction of ovulation by 9%.

Unidentified factors may play a role in determining endometrial receptivity during the window of implantation in normal condition or in CCF patients with impaired endometrial receptivity after long term exposure to CC. In the present study, CRA patients are more likely to develop multiple follicle growth thus more chance for cancellation of the exogenous FSH-induced cycle. Moreover, some reports indicate impaired chance of conception in these women due to detrimental effect of hyperandrogenemia on oocyte quality. To our surprise, CRA patients exhibit comparable chance of conception during exogenous gonadotropins compared to CCF who are more likely to be normoandrogenic.

We did not observe any detrimental effect of augmented serum LH level for the chances of ongoing pregnancy. Initial serum LH level predicts chances for multiple follicle development but not ongoing pregnancy in the present study. We were unable to observe any significant increase in the chance of spontaneous miscarriages in patients with an elevated initial serum LH level. Reports in the literature are contradictory. Our finding is in contrast with previous reports indicating a higher percentage of miscarriages in these patients. However, on the basis of these observations one may not have hard conclusion due to the limited number of miscarriages in the present study. Initial serum insulin, insulin/glucose ratio, or inhibin B had no predictive value for prediction of chances for ongoing pregnancy during exogenous gonadotropin induction of ovulation. Moreover, TMC does not predict chances of conception during exogenous FSH induced cycles. This is agreement with our report on predictors of CC ovulation induction outcome (Imani *et al.*, 1999).

In conclusion, the present longitudinal follow-up study suggests that clomiphene resistant anovulatory women who exhibit an elevated serum AD level in combination with an augmented number of follicles prior to ovarian stimulation are at high risk for multiple follicle development during gonadotropin ovulation induction applying a step-down regimen. They exhibit a higher chance for cancellation of the FSH-induced cycle. Applying a low-dose step-up cycle prior to step-down cycle, the starting FSH dose for step-down cycle was adjusted. Moreover, applying strict cancellation criteria the complication rates were minimised. However, CCR of 65% was reached within 8 stimulated cycles, which is comparable to CCR reported in the literature. We report merely singleton pregnancies. Moreover, young CRA or CCF women who exhibit an elevated serum IGF-I level and normal T level are at high risk to reach an ongoing pregnancy. CRA patients are at high risk for cancellation of the cycle due to multiple follicle growth. CRA patients are more likely to exhibit multifollicular development during ovarian stimulation applying exogenous FSH due to hyperandrogenemia. However, they exhibit a comparable chance of reaching

ongoing pregnancy compared to CCF. Patients, who failed to conceive despite ovulatory CC-induced cycles, benefit a good chance of pregnancy during exogenous FSH induction of ovulation. This indicates that assisted reproduction techniques should not be introduced in women who ovulated but failed to conceive following CC. Gonadotropins should be considered as ovulation induction strategy with high chances of pregnancy particularly in young, CRA or CCF patients who exhibit an elevated level of IGF-I. Only those women who are more likely to fail to conceive due to impaired fertility chances (advanced age or high chance of hyperresponse during exogenous FSH stimulation), should be devised to benefit from alternative treatment options such as assisted reproductive techniques. Prospective studies should be performed to evaluate significance of these parameters for prediction of treatment outcome following assisted reproductive techniques such as *in vitro* fertilisation in those patients who failed to conceive during exogenous gonadotropin induction of ovulation applying a modified step-down regimen.

Chapter IV

General discussion and conclusions

Studies presented in this thesis were set up attempting to identify clinical, endocrine and ultrasound features upon initial screening to predict outcome of ovulation induction in normogonadotropic anovulatory infertile women. This approach in a very heterogeneous group of patients might render clinical intervention more cost effective by allowing individualized treatment strategies. This line of thinking requiers a paradigm shift from diagnosis to prognosis. The search for etiological factors in cross sectional studies is inherently weak. This new longitudinal approach may provide useful information regarding the pathophysiology of underlying ovarian abnormalities in this heterogeneous patient population. I.e. factors involved in the response of ovaries to stimulation by endogenous or exogenous FSH may indeed be directly related to ovarian dysfunction. In this respect, ovulation induction can be considered an extended challange test.

Currently, CC represents the first line treatment strategy for all patients with normogonadotropic anovulatory infertility. CC is safe and convenient with limited chances for complications such as multiple pregnancy or OHSS. However, only 38% of treated women may conceive following CC resulting in live birth which is substantially less than generally assumed. As indicated earlier, a great majority of patients do not respond to maximum dose of CC administered. It may represent a distinct step forward if patients remaining anovulatory following CC therapy or patients with extreme low chance for live birth despite ovulatory CC cycles could be identified prior to initiation of therapy. Ineffective and time-consuming CC medication could be prevented and alternative treatment modalities could be applied. This may be helpful, particularly for women of advanced reproductive age.

For the first time, our group has focused on ovulation and conception in separate steps. Two multivariate prediction models have been introduced in the treatment of anovulatory infertility applying CC. The first multivariate model consisting of FAI, BMI, cycle history, and mean ovarian volume predicts chances of remaining anovulatory following CC (Imani *et al.*, 1998). The second model consisting of age and cycle history predicts chances for conception only in women who respond to CC (Imani *et al.*, 1999). These two models were subsequently implemented in a nomogram. The first part of the nomogram, depicted in left side of the nomogram chart, is to predict the chances of ovulation following CC applying three initial screening characteristics, FAI, BMI, and cycle history. As indicated earlier, FAI is the most significant endocrine marker of ovarian dysfunction and for the prediction of ovarian response following CC. External validation of the present nomogram is mandatory to allow the definition of a clear cut-off level in the chances for live birth after CC for the decision making in routine daily clinical practice. These patients could be advised to refrain from CC therapy and start with an alternative first line treatment modality such as weight reduction, exogenous

gonadotropins, insulin sensitizing hormones, or *in vitro* fertilization, particularly in women of advanced age. This would render CC ovulation induction more patient-tailored and could improve overall cost effectiveness.

Complications of induction of ovulation applying exogenous gonadotropins in the group of normogonadotropic clomiphene resistent oligomenorrheic or amenorrheic infertile women are a cause of major concern. These complications are mainly OHSS and multiple pregnancy, which seem to be correlated with the dose regimens applied (van Santbrink *et al.*, 1997a), late follicular phase FSH accumulation and / or multifollicular development (Fauser *et al.*, 1997). The initial starting dose of FSH for any given patient is a crucial point since it might be inapropriately too high or too low, in the majority of patients who exhibit a too low or a too high FSH threshold level. In these patients, stimulation should be cancelled due to hyperresponse. Currently, this represents the major drawback of the step-down approach (Fauser *et al.*, 1997). By choosing the appropriate starting FSH dose chances of OHSS or multiple gestations may be restricted.

As second step in the present study we focused on prediction of FSH response dose which reflects FSH threshold level. The individual FSH response dose in anovulatory infertile women can be predicted using BMI, ovarian response during preceding CC medication, and serum free IGF-I and initial FSH concentrations. The possibility to predict the individual FSH threshold may increase safety, efficiency and patient convenience of low-dose regimens for gonadotropin induction of ovulation by the proper assessment of the appropriate starting dose for a given patient. This may represent a distinct step forward in the clinic. Time-consuming low dose increments may be overcome by starting with a high dose in women with an augmented FSH threshold. In contrast, starting with an individualized dose in step-down regimens may reduce chances for hyperresponse in women with a low FSH threshold. FSH threshold may represent the severity of ovarian abnormalities in these patients. Indeed, ovarian response to preceding CC medication predicts ovarian response during subsequent stimulation by exogenous FSH. This finding confirms the concept previously proposed by our group indicating that CRA patients may suffer from a more serious ovarian abnormality which is more resistant to stimulation by FSH (Imani *et al.*, 1998; Imani *et al.*, 2000). Patients who ovulated following CC but failed to conceive exhibit a lower FSH threshold and may subsequently require lower dose of exogenous FSH to reach sufficient ovarian stimulation.

Next, a model has been introduced to predict the chance of multiple follicular development in gonadotropin induced cycles applying decremental dose regimen. Multivariate analyses revealed a final model including initial serum AD level, being a CRA and the total number of follicles prior to initiation of stimulation.

Further well-designed prospective randomised studies are required to investigate whether alternative treatment options such as "ovarian drilling" or "insulin sentising agents" are cost effective in patients predicted applying this model. In the present study a CCR of %65 have been reached within eight consecutive gonadotropin ovulation induction cycles.

Overlooking the findings dicussed in this thesis the following recommendations for future studies can be made:

- Extensive validation of these prediction models should be performed in other, independent patient populations.

- Further follow up studies should be performed in the current series of patients after unsuccessful ovulation induction undergoing in vitro fertilization. Can ovarian response to hyperstimulation and chances for success and complications be predicted? (Mulders *et al.*, In preparation)

- We should go back to the literature and investigate whether confirmation of the importance of factors identified in our multi-variate analysis for ovarian pathophysiology and outcome of ovulation induction can be found. (Mulders *et al.*, In preparation)

- A flow chart for the health economic approach of classical ovulation induction may be designed on the basis of the current findings. (Eijkemans *et al.*, In preparaton)

- A multi-variate analysis should be performed for the prediction of classical ovulation induction outcome (CC and FSH combined). (Eijkemans *et al.*, In preparation)

- Alternative treatment approaches may be explored in women with predicted low success chances with conventional ovulation induction, such as:
  - FSH as first treatment in women with low chances for life birth following CC treatment.
  - The application of insulin sensitizing agents or aromatase inhibitors as adjunctive or first line therapy in low chance patients.
  - The surgical/laser approaches as adjunctive or first line tretament in low chance patients
  - In vitro fertilisation as first line treatment in low chance patients.

References

Adashi, E. Y. (1996) Ovulation Induction: Clomiphene Citrate. Adashi, E. Y., Rock, J. A., and Rosenwaks, Z. *Reproductive Endocrinology, Survey, and Technology.* Lippincott-Raven, Philadelphia: 1182-1206

Agarwal, S. K., Vogel, K., Weitsman, S. R., and Magoffin, D. A. (1999) Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. *J Clin Endocrinol Metab,* **84:** 1072-1076

Agrawal, R., Conway, G., Sladkevicius, P., Tan, S. L., Engmann, L., Payne, N., Bekir, J., Campbell, S., and Jacobs, H. (1998a) Serum vascular endothelial growth factor and Doppler blood flow velocities in in vitro fertilization: relevance to ovarian hyperstimulation syndrome and polycystic ovaries. *Fertil Steril,* **70:** 651-658

Agrawal, R., Sladkevicius, P., Engmann, L., Conway, G. S., Payne, N. N., Bekis, J., Tan, S. L., Campbell, S., and Jacobs, H. S. (1998b) Serum vascular endothelial growth factor concentrations and ovarian stromal blood flow are increased in women with polycystic ovaries. *Hum Reprod,* **13:** 651-655

Anderson, R. A., Groome, N. P., and Baird, D. T. (1998) Inhibin A and inhibin B in women with polycystic ovarian syndrome during treatment with FSH to induce mono-ovulation. *Clin Endocrinol (Oxf),* **48:** 577-584

Axelrod, L. R. and Goldzieher, J. W. (1962) The polycystic ovary. III. Steroid biosynthesis in normal and polycystic ovarian tissue. *J Clin Endocrinol Metab,* **22:** 431-440

Balen, A. H., Braat, D. D., West, C., Patel, A., and Jacobs, H. S. (1994) Cumulative conception and live birth rates after the treatment of anovulatory infertility: safety and efficacy of ovulation induction in 200 patients. *Hum Reprod,* **9:** 1563-1570

Ben, Rafael, Levy, T., and Schoemaker, J. (1995) Pharmacokinetics of follicle-stimulating hormone: clinical significance. *Fertil Steril,* **63:** 689-700

Berga, S. L. and Daniels, T. L. (1997) Can Polycystic Ovary Syndrome exist without concomitant hypothalamic dysfunction? *Seminars in Reproductive Endocrinology,* **15:** 169-175

Brown, J. B. (1978) Pituitary control of ovarian function—concepts derived from gonadotrophin therapy. *Aust N Z J Obstet Gynaecol*, **18**: 46-54

Brzechffa, P. R., Jakimiuk, A. J., Agarwal, S. K., Weitsman, S. R., Buyalos, R. P., and Magoffin, D. A. (1996) Serum immunoreactive leptin concentrations in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*, **81**: 4166-4169

Buckler, H. M., Critchley, H. O., Cantrill, J. A., Shalet, S. M., Anderson, D. C., and Robertson, W. R. (1993) Efficacy of low dose purified FSH in ovulation induction following pituitary desensitization in polycystic ovarian syndrome. *Clin Endocrinol (Oxf)*, **38**: 209-217

Burghen, G. A., Givens, J. R., and Kitabchi, A. E. (1980) Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. *J Clin Endocrinol Metab*, **50**: 113-116

Butzow, T. L., Kettel, L. M., and Yen, S. S. (1995) Clomiphene citrate reduces serum insulin-like growth factor I and increases sex hormone-binding globulin levels in women with polycystic ovary syndrome. *Fertil Steril*, **63**: 1200-1203

Buvat, J., Buvat, Herbaut, Marcolin, G., Dehaene, J. L., Verbecq, P., and Renouard, O. (1989) Purified follicle-stimulating hormone in polycystic ovary syndrome: slow administration is safer and more effective. *Fertil Steril*, **52**: 553-559

Callahan, T. L., Hall, J. E., Ettner, S. L., Christiansen, C. L., Greene, M. F., and Crowley, W. F. J. (1994) The economic impact of multiple-gestation pregnancies and the contribution of assisted-reproduction techniques to their incidence. *The New England Journal of Medicine*, **331**: 244-249

Carantoni, M., Abbasi, F., Azhar, S., Schaaf, P., and Reaven, G. M. (1999) Can changes in plasma insulin concentration explain the variability in leptin response to weight loss in obese women with normal glucose tolerance? *J Clin Endocrinol Metab*, **84**: 869-872

Cataldo, N. A. and Giudice, L. C. (1992) Insulin-like growth factor binding protein profiles in human ovarian follicular fluid correlate with follicular functional status. *J Clin Endocrinol Metab*, **74**: 821-829

Cataldo, N. A. (1997) Insulin-like growth factor binding proteins: do they play a role in polycystic ovary syndrome? *Semin Reprod Endocrinol*, **15:** 123-136

Cheng, J. M., Hiemstra, J. L., Schneider, S. S., Naumova, A., Cheung, N. K., Cohn, S. L., Diller, L., Sapienza, C., and Brodeur, G. M. (1993) Preferential amplification of the paternal allele of the N-myc gene in human neuroblastomas. *Nat Genet*, **4:** 191-194

Chong, A. P., Rafael, R. W., and Forte, C. C. (1986) Influence of weight in the induction of ovulation with human menopausal gonadotropin and human chorionic gonadotropin. *Fertil Steril*, **46:** 599-603

Clark, A. M., Ledger, W., Galletly, C., Tomlinson, L., Blaney, F., Wang, X., and Norman, R. J. (1995) Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. *Hum Reprod*, **10:** 2705-2712

Clark, A. M., Thornley, B., Tomlinson, L., Galletley, C., and Norman, R. J. (1998) Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. *Hum Reprod*, **13:** 1502-1505

Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J. M., Basdevant, A., Bougneres, P., Lebouc, Y., Froguel, P., and Guy-Grand, B. (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, **392:** 398-401

Cohen, B., Novick, D., and Rubinstein, M. (1996) Modulation of insulin activities by leptin. *Science*, **274:** 1185-1188

Collins, J. A., Burrows, E. A., and Wilan, A. R. (1995) The prognosis for live birth among untreated infertile couples. *Fertil Steril*, **64:** 22-28

Cox, D. R. (1972) Regression models and life tables. *J R Statist Soc*, **B34:** 187-220

Dale, O., Tanbo, T., Lunde, O., and Abyholm, T. (1993) Ovulation induction with low-dose follicle-stimulating hormone in women with the polycystic ovary syndrome. *Acta Obstet Gynecol Scand*, **72:** 43-46

Dale, P. O., Tanbo, T., Haug, E., and Abyholm, T. (1992) Polycystic ovary syndrome: low-dose follicle stimulating hormone administration is a safe stimulation regimen even in previous hyper-responsive patients. *Hum Reprod*, **7:** 1085-1089

de Jong, F. H., Baird, D. T., and van der Molen HJ. (1974) Ovarian secretion rates of oestrogens, androgens and progesterone in normal women and in women with persistent ovarian follicles. *Acta Endocrinologica*, **77:** 575-587

Devoto, E., Aravena, L., and Gaete, X. (1998) [Has oligomenorrhea a pathological meaning? The importance of this symptom in internal medicine]. *Rev Med Chil*, **126:** 943-951

Dor, J., Itzkowic, D. J., Mashiach, S., Lunenfeld, B., and Serr, D. M. (1980) Cumulative conception rates following gonadotropin therapy. *Am J Obstet Gynecol*, **136:** 102-105

Dunaif, A., Mandeli, J., Fluhr, H., and Dobrjansky, A. (1988) The impact of obesity and chronic hyperinsulinemia on gonadotropin release and gonadal steroid secretion in the polycystic ovary syndrome. *J Clin Endocrinol Metab*, **66:** 131-139

Dunaif, A., Givens JR, Haseltine F, and Merriam GR (eds) (1992) The Polycystic Ovary Syndrome. Blackwell Scientific, Cambridge, MA.

Dunaif, A., Scott, D., Finegood, D., Quintana, B., and Whitcomb, R. (1996) The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. *J Clin Endocrinol Metab*, **81:** 3299-3306

Dunaif, A. (1997) Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev*, **18:** 774-800

Eden, J. A., Place, J., Carter, G. D., Alaghband-Zadeh, J., and Pawson, M. E. (1989) The role of chronic anovulation in the polycystic ovary syndrome: normalization of sex-hormone-binding globulin levels after clomiphene- induced ovulation. *Clin.Endocrinol.(Oxf.)*, **30:** 323-332

Eimers, J. M., te Velde, E. R., Gerritse, R., Vogelzang, E. T., Looman, C. W., Habbema, and JD. (1994) The prediction of the chance to conceive in subfertile couples. *Fertil Steril*, **61**: 44-52

Emperaire, J. C., Gauzere-Soumireu, E., and Audebert, A. J. (1982) Female fertility and donor insemination. *Fertil Steril*, **37**: 90-93

Farquhar, C., Vandekerckhove, P., Arnot, M., and Lilford, R. (2000) Laparoscopic "drilling" by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. *Cochrane Database Syst Rev*, CD001122-CD001122

Fauser, B. C., Pache, T. D., Lamberts, S. W., Hop, W. C., de Jong, F. H., and Dahl, K. D. (1991) Serum bioactive and immunoreactive luteinizing hormone and follicle-stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovarian disease. *J Clin Endocrinol Metab*, **73**: 811-817

Fauser, B. C. and de Jong, F. H. (1993a) Gonadotropins in polycystic ovarian syndrome. *Annals of the New York Academy of Sciences*, **687**: 150-161

Fauser, B. C., Donderwinkel, P., and Schoot, D. C. (1993b) The step-down principle in gonadotrophin treatment and the role of GnRH analogues. *Baillieres Clinical Obstetrics & Gynaecology*, **7**: 309-330

Fauser, B. C. (1993c) Clinical use of gonadotropins in women. Bouchard, P., Caraty, A., Coelingh Bennink, H. J. T., and Pavlou, S. N. *GnRH, GnRH analogs, Gonadotropins, and gonadal peptides*. Parthenon Publishing, London, UK, 467-479

Fauser, B. C. (1994) Observations in favor of normal early follicle development and disturbed dominant follicle selection in polycystic ovary syndrome. *Gynecological Endocrinology*, **8**: 75-82

Fauser, B. C. and Van Heusden, A. M. (1997) Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr Rev*, **18**: 71-106

Fauser, B. C., Conway, G. S., and Franks, S. (1999) Genetics of female reproductive dysfunction. Fauser, B. C., Rutherford, A. J., Strauss, J. F. 3d, and Steirteghem, A. C. *Molecular Biology in Reproductive Medicine*. Parthenon Publishing. London.

Fluker, M. R., Wang, I. Y., and Rowe, T. C. (1996) An extended 10-day course of clomiphene citrate (CC) in women with CC-resistant ovulatory disorders. *Fertil Steril*, **66:** 761-764

Forster, I., Gu, H., and Rajewsky, K. (1988) Germline antibody V regions as determinants of clonal persistence and malignant growth in the B cell compartment. *EMBO J*, **7:** 3693-3703

Franks, S. and Gilling-Smith, C. (1994) Advances in induction of ovulation. *Current Opinion in Obstetrics & Gynecology*, **6:** 136-140

Franks, S. (1995) Polycystic ovary syndrome. *The New England Journal of Medicine*, **333:** 853-861

Franks, S., Mason, H., and Willis, D. (1998) Disorders of folliculogenesis in polycystic ovary syndrome. Filicori, M. and Flamigni, C. *Ovulation induction update '98*. The parthenon publishing group., 37-40

Garcia, J., Jones, G. S., and Wentz, A. C. (1977) The use of clomiphene citrate. *Fertil Steril*, **28:** 707-717

Ginsburg, J. and Hardiman, P. (1991) Ovulation induction with human menopausal gonadotropins—a changing scene. *Gynecol Endocrinol*, **5:** 57-78

Giudice, L. C. (1992) Insulin-like growth factors and ovarian follicular development. *Endocr Rev*, **13:** 641-669

Giudice, L. C., Morales, A. J., and Yen, S. S. (1996) Growth factors and polycystic ovarian syndrome. *Semin Reprod Endocrinol*, **14:** 203-208

Givens, J. R., Andersen, R. N., Wiser, W. L., and Fish, S. A. (1974) Dynamics of suppression and recovery of plasma FSH, LH, androstenedione and testosterone in polycystic ovarian disease using an oral contraceptive. *J Clin Endocrinol Metab*, **38:** 727-735

Gleicher, N., Oleske, D. M., Tur-Kaspa, I., Vidali, A., and Karande, V. (2000) Reducing the risk of high-order multiple pregnancy after ovarian stimulation with gonadotropins. *The New England Journal of Medicine*, **343:** 2-7

Golan, A., Ron, el, Herman, A., Soffer, Y., Weinraub, Z., and Caspi, E. (1989) Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol Surv*, **44**: 430-440

Goldzieher, J. W. and Axelrod, L. R. (1963) Clinical and biochemical features of polycystic ovarian disease. *Fertil Steril*, **14**: 631-653

Gorlitsky, G. A., Kase, N. G., and Speroff, L. (1978) Ovulation and pregnancy rates with clomiphene citrate. *Obstet Gynecol*, **51**: 265-269

Greenblatt, R. B., Barfield, W. E., Jungck, E. C., and Ray, A. W. (1961) Induction of ovulation with MRL/41. *J Am Med Assoc*, **178**: 127-130

Groome, N. P., Illingworth, P. J., O'Brien, M., Pai, R., Rodger, F. E., Mather, J. P., and McNeilly, A. S. (1996) Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab*, **81**: 1401-1405

Gysler, M., March, C. M., Mishell, D. R., Jr., and Bailey, E. J. (1982) A decade's experience with an individualized clomiphene treatment regimen including its effect on the postcoital test. *Fertil Steril*, **37**: 161-167

Hamilton-Fairley, D., Kiddy, D., Watson, H., Paterson, C., and Franks, S. (1992) Association of moderate obesity with a poor pregnancy outcome in women with polycystic ovary syndrome treated with low dose gonadotrophin. *Br J Obstet Gynaecol*, **99**: 128-131

Hamilton-Fairley, D., Kiddy, D., Watson, H., Sagle, M., and Franks, S. (1991) Low-dose gonadotrophin therapy for induction of ovulation in 100 women with polycystic ovary syndrome. *Hum Reprod*, **6**: 1095-1099

Hammond, M. G., Halme, J. K., and Talbert, L. M. (1983) Factors affecting the pregnancy rate in clomiphene citrate induction of ovulation. *Obstet Gynecol*, **62**: 196-202

Harrell, F. E., Jr., Lee, K. L., and Mark, D. B. (1996) Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Statistics in Medicine*, **15**: 361-387

Healy, D. L., Kovacs, G. T., Pepperell, R. J., and Burger, H. G. (1980) A normal cumulative conception rate after human pituitary gonadotropin. *Fertil Steril*, **34:** 341-345

Healy, D. L. and Burger, H. G. (1983) Serum follicle-stimulating hormone, luteinizing hormone, and prolactin during the induction of ovulation with exogenous gonadotropin. *J Clin Endocrinol Metab*, **56:** 474-478

Herman, A., Ron, el, Golan, A., Soffer, Y., Bukovsky, I., and Caspi, E. (1993) Overstimulated cycles under low-dose gonadotrophins in patients with polycystic ovary syndrome: characterization and management. *Hum Reprod*, **8:** 30-34

Hollmann, M., Runnebaum, B., and Gerhard, I. (1996) Effects of weight loss on the hormonal profile in obese, infertile women. *Hum Reprod*, **11:** 1884-1891

Homburg, R., Armar, N. A., Eshel, A., Adams, J., and Jacobs, H. S. (1988) Influence of serum luteinising hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. *British Medical Journal*, **297:** 1024-1026

Homburg, R., Levy, T., and Ben-Rafael, Z. (1995) A comparative prospective study of conventional regimen with chronic low-dose administration of follicle-stimulating hormone for anovulation associated with polycystic ovary syndrome. *Hum Reprod*, **63:** 729-733

Hosmer, D. W. and Lemeshow, S. (1989) Applied Logistic Regression. *Wiley, New York*

Howles, C. M., Macnamee, M. C., Edwards, R. G., Goswamy, R., and Steptoe, P. C. (1986) Effect of high tonic levels of luteinising hormone on outcome of in-vitro fertilisation. *Lancet*, **2:** 521-522

Hull, M. G. (1987) Epidemiology of infertility and polycystic ovarian disease: endocrinological and demographic studies. *Gynecological Endocrinology*, **1:** 235-245

Hull, M. G. (1991) Gonadotropin therapy in anovulatory infertility. Howles, C. M.Alden Press, Oxford, UK, 56-61

Hull, M. G., Glazener, C. M., Kelly, N. J., Conway, D. I., Foster, P. A., Hinton, R. A., Coulson, C., Lambert, P. A., Watt, E. M., and Desai, K. M. (1985) Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)*, **291:** 1693-1697

Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (1998) Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, **83:** 2361-2365

Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (1999) Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, **84:** 1617-1622

Imani, B., Eijkemans, M. J., de Jong, F. H., Payne, N. N., Bouchard, P., Giudice, L. C., and Fauser, B. C. (2000) Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, **85:** 676-682

Imani, B., Eijkemans, M. J., Faessen, G., Bouchard, P., Giudice, L. C., and Fauser, B. C. (2002a) Prediction of the individual follicle-stimulating hormone threshold for gonadotrophin induction of ovulation in anovulatory infertility: An approach to minimize multiple gestation, ovarian hyperstimulation, and treatment expense. *Fertil Steril*, **77:** 83-90

Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (2002b) A nomogram to predict live birth probability after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *Fertil Steril*, **77:** 91-97

Insler, V. (1988) Gonadotropin therapy: new trends and insights. *Int J Fertil*, **33:** 85-97

Jacobson, A., Marshall, J. R., Ross, G. T., and Cargille, C. M. (1968) Plasma gonadotropins during clomiphene induced ovulatory cycles. *Am J Obstet Gynecol*, **102:** 284-290

Kahn, C. R., Flier, J. S., Bar, R. S., Archer, J. A., Gorden, P., Martin, M. M., and Roth, J. (1976) The syndromes of insulin resistance and acanthosis nigricans. Insulin-receptor disorders in man. *The New England Journal of Medicine*, **294:** 739-745

Kaplan, E. L. and Meier, P. (1958) Nonparametric estimation from incomplete observations. *J Am Statist Assoc*, **53:** 457-481

Kelly, A. C. and Jewelewicz, R. (1990) Alternate regimens for ovulation induction in polycystic ovarian disease. *Fertil Steril*, **54:** 195-202

Kerin, J. F., Liu, J. H., Phillipou, G., and Yen, S. S. (1985) Evidence for a hypothalamic site of action of clomiphene citrate in women. *J Clin Endocrinol Metab*, **61:** 265-268

Kiddy, D. S., Hamilton-Fairley, D., Seppala, M., Koistinen, R., James, V. H., Reed, M. J., and Franks, S. (1989) Diet-induced changes in sex hormone binding globulin and free testosterone in women with normal or polycystic ovaries: correlation with serum insulin and insulin-like growth factor-I. *Clin Endocrinol (Oxf)*, **31:** 757-763

Kocak, M, Caliskan, E, Simsir, C, and Haberal, A (2002) Metformin therapy improves ovulatory rates, cervical scores, and pregnancy rates in clomiphene citrate resistant women with polycystic ovary syndrome. *Fertil Steril*, **77:** 101-106

Kousta, E., White, D. M., and Franks, S. (1997) Modern use of clomiphene citrate in induction of ovulation. *Hum Reprod Update*, **3:** 359-365

Lahlou, N., Landais, P., De Boissieu, D., and Bougneres, P. F. (1997) Circulating leptin in normal children and during the dynamic phase of juvenile obesity: relation to body fatness, energy metabolism, caloric intake, and sexual dimorphism. *Diabetes*, **46:** 989-993

Laughlin, G. A., Morales, A. J., and Yen, S. S. (1997) Serum leptin levels in women with polycystic ovary syndrome: the role of insulin resistance/hyperinsulinemia. *J Clin Endocrinol Metab*, **82:** 1692-1696

Legro, R. S., Spielman, R., Urbanek, M., Driscoll, D., Strauss, J. F., and Dunaif, A. (1998) Phenotype and genotype in polycystic ovary syndrome. *Recent Prog Horm Res*, **53:** 217-256

LeRoith, D., Werner, H., Beitner-Johnson, D., and Roberts, C. T. J. (1995) Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocr Rev*, **16:** 143-163

Lobo, R. A., Granger, L. R., Davajan, V., and Mishell, D. R. J. (1982a) An extended regimen of clomiphene citrate in women unresponsive to standard therapy. *Fertil Steril*, **37:** 762-766

Lobo, R. A., Gysler, M., March, C. M., Goebelsmann, U., and Mishell, D. R. J. (1982b) Clinical and laboratory predictors of clomiphene response. *Fertil Steril*, **37:** 168-174

Lunenfeld, B. and Insler, V. (1974) Classification of amenorrhoeic states and their treatment by ovulation induction. *Clinical Endocrinology*, **3:** 223-237

Lunenfeld, B., Mashiach, S., and Blankstein, J. (1985) Induction of ovulation with gonadotrohins. Shearman, R. P. *Clinical Reproductive Endocrinology*. Churchill Livingstone, Edinburgh, 523-533

MacGregor, A. H., Johnson, J. E., and Bunde, C. A. (1968) Further clinical experience with clomiphene citrate. *Fertil Steril*, **19:** 616-622

Mallya, S., Abdulla, U. A., and Davis, J. C. (1993) Measurement of serum levels of exogenously administered FSH and LH during ovulation induction therapy. *Gynecol Endocrinol*, **7:** 167-171

Mannaerts, B., Shoham, Z., Schoot, D., Bouchard, P., Harlin, J., Fauser, B., Jacobs, H., Rombout, F., and Coelingh, Bennink (1993) Single-dose pharmacokinetics and pharmacodynamics of recombinant human follicle-stimulating hormone (Org 32489*) in gonadotropin-deficient volunteers. *Fertil Steril*, **59:** 108-114

Marrs, R. P., Vargyas, J. M., and March, C. M. (1983) Correlation of ultrasonic and endocrinologic measurements in human menopausal gonadotropin therapy. *Am J Obstet Gynecol*, **145:** 417-421

McClure, N., McQuinn, B., McDonald, J., Kovacs, G. T., Healy, D. L., and Burger, H. G. (1992) Body weight, body mass index, and age: predictors of menotropin dose and cycle outcome in polycystic ovarian syndrome? *Fertil Steril*, **58:** 622-624

Messinis, I. E. and Templeton, A. A. (1990) The importance of follicle-stimulating hormone increase for folliculogenesis. *Hum Reprod*, **5**: 153-156

Messinis, I. E., Milingos, S. D., Alexandris, E., Kariotis, I., Kollios, G., and Seferiadis, K. (1999) Leptin concentrations in normal women following bilateral ovariectomy. *Hum Reprod*, **14**: 913-918

Minakami, H., Abe, N., Izumi, A., and Tamada, T. (1988) Serum luteinizing hormone profile during the menstrual cycle in polycystic ovarian syndrome. *Fertil Steril*, **50**: 990-992

Mitwally, M. F. and Casper, R. F. (2001) Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. *Fertil Steril*, **75**: 305-309

Miyake, A., Tasaka, K., Sakumoto, T., Kawamura, Y., Nagahara, Y., and Aono, T. (1983) Clomiphene citrate induces luteinizing hormone release through hypothalamic luteinizing hormone-releasing hormone in vitro. *Acta Endocrinologica*, **103**: 289-292

Mizunuma, H., Takagi, T., Honjyo, S., Ibuki, Y., and Igarashi, M. (1990) Clinical pharmacodynamics of urinary follicle-stimulating hormone and its application for pharmacokinetic simulation program. *Fertil Steril*, **53**: 440-445

Neeley, W. E. (1972) Simple automated determination of serum or plasma glucose by a hexokinase-glucose-6 -phosphate dehydrogenase method. *Clinical Chemistry*, **18**: 509-515

Nelson, V. L., Legro, R. S., Strauss, J. F., and McAllister, J. M. (1999) Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. *Mol Endocrinol*, **13**: 946-957

Nestler, J. E. (1997a) Role of hyperinsulinemia in the pathogenesis of the polycystic ovary syndrome, and its clinical implications. *Seminars in Reproductive Endocrinology*, **15**: 111-122

Nestler, J. E. and Jakubowicz, D. J. (1997b) Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. *J Clin Endocrinol Metab*, **82**: 4075-4079

Nestler, J. E., Jakubowicz, D. J., de Vargas, A. F., Brik, C., Quintero, N., and Medina, F. (1998) Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. *J Clin Endocrinol Metab*, **83**: 2001-2005

Nestler, J. E., Powers, L. P., Matt, D. W., Steingold, K. A., Plymate, S. R., Rittmaster, R. S., Clore, J. N., and Blackard, W. G. (1991) A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab*, **72**: 83-89

Oelsner, G., Serr, D. M., Mashiach, S., Blankstein, J., Snyder, M., and Lunenfeld, B. (1978) The study of induction of ovulation with menotropins: analysis of results of 1897 treatment cycles. *Fertil Steril*, **30**: 538-544

Ombelet, W., Bosmans, E., Janssen, M., Cox, A., Vlasselaer, J., Gyselaers, W., Vandeput, H., Gielen, J., Pollet, H., Maes, M., Steeno, O., and Kruger, T. (1997) Semen parameters in a fertile versus subfertile population: a need for change in the interpretation of semen testing. *Hum Reprod*, **12**: 987-993

Opsahl, M. S., Robins, E. D., O'Connor, D. M., Scott, R. T., and Fritz, M. A. (1996) Characteristics of gonadotropin response, follicular development, and endometrial growth and maturation across consecutive cycles of clomiphene citrate treatment. *Fertil Steril*, **66**: 533-539

Pache, T. D., Wladimiroff, J. W., de Jong, F. H., Hop, W. C., and Fauser, B. C. (1990) Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. *Fertil Steril*, **54**: 638-642

Pache, T. D., Hop, W. C., Wladimiroff, J. W., Schipper, J., and Fauser, B. C. (1991a) Transvaginal sonography and abnormal ovarian appearance in menstrual cycle disturbances. *Ultrasound Med Biol*, **17**: 589-593

Pache, T. D., Chadha, S., Gooren, L. J., Hop, W. C., Jaarsma, K. W., Dommerholt, H. B., and Fauser, B. C. (1991b) Ovarian morphology in long-term androgen-treated female to male transsexuals. A human model for the study of polycystic ovarian syndrome? *Histopathology*, **19**: 445-452

Pache, T. D., Hop, W. C., de Jong, F. H., Leerentveld, R. A., van Geldorp, H., Van de, Kamp, T. M., Gooren, L. J., and Fauser, B. C. (1992a) 17 beta-Oestradiol, androstenedione and inhibin levels in fluid from individual follicles of normal and polycystic ovaries, and in ovaries from androgen treated female to male transsexuals. *Clin Endocrinol (Oxf)*, **36:** 565-571

Pache, T. D., Wladimiroff, J. W., Hop, W. C., and Fauser, B. C. (1992b) How to discriminate between normal and polycystic ovaries: transvaginal US study. *Radiology*, **183:** 421-423

Pache, T. D., de Jong, F. H., Hop, W. C., and Fauser, B. C. (1993) Association between ovarian changes assessed by transvaginal sonography and clinical and endocrine signs of the polycystic ovary syndrome. *Fertil Steril*, **59:** 544-549

Pasquali, R. and Casimirri, F. (1993) The impact of obesity on hyperandrogenism and polycystic ovary syndrome in premenopausal women. *Clin Endocrinol (Oxf)*, **39:** 1-16

Polson, D. W., Kiddy, D. S., Mason, H. D., and Franks, S. (1989) Induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome: the difference between responders and nonresponders. *Fertil Steril*, **51:** 30-34

Poretsky, L. (1991) On the paradox of insulin-induced hyperandrogenism in insulin-resistant states. *Endocr Rev*, **12:** 3-13

Poretsky, L., Chandrasekher, Y. A., Bai, C., Liu, H. C., Rosenwaks, Z., and Giudice, L. C. (1996) Insulin receptor mediates inhibitory effect of insulin, but not of insulin-like growth factor (IGF)-I, on IGF binding protein 1 (IGFBP-1) production in human granulosa cells. *J Clin Endocrinol Metab*, **81:** 493-496

Poretsky, L., Cataldo, N. A., Rosenwaks, Z., and Giudice, L. C. (1999) The insulin-related ovarian regulatory system in health and disease. *Endocr Rev*, **20:** 535-582

Preleviç, G. M., Wurzburger, M. I., and Balint, Peric (1990) LH pulsatility and response to a single s.c. injection of buserelin in polycystic ovary syndrome. *Gynecol Endocrinol*, **4:** 1-13

Regan, L., Owen, E. J., and Jacobs, H. S. (1990) Hypersecretion of luteinising hormone, infertility, and miscarriage. *Lancet*, **336:** 1141-1144

Rouru, J., Anttila, L., Koskinen, P., PenttilS, T. A., Irjala, K., Huupponen, R., and Koulu, M. (1997) Serum leptin concentrations in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*, **82**: 1697-1700

Rowe, P. J., Combaire, F. H., Hargreave, T. B., and Mellows, H. (2001) WHO manual for the standardized investigation and diagnosis of the infertile couple. Rowe, P. J., Combaire, F. H., Hargreave, T. B., and Mellows, H. *Female partner.* Cambridge: Press syndicate of the University of Cambridge, 2000, 40-67

Rubin, D. B. (1978) Multiple imputation in sample surveys - a phenomenological Baysian approach to nonresponse. Proceedings of the survey Research methods section of the American Statistical Association. ., 20-34

San Roman, G. A. and Magoffin, D. A. (1992) Insulin-like growth factor binding proteins in ovarian follicles from women with polycystic ovarian disease: cellular source and levels in follicular fluid. *J Clin Endocrinol Metab*, **75**: 1010-1016

Sattar, N., Hopkinson, ZE, and Greer, IA (1998) Insulin-sensitising agents in polycystic-ovary syndrome. *Lancet*, **351**: 305-307

Schenker, J. G., Yarkoni, S., and Granat, M. (1981) Multiple pregnancies following induction of ovulation. *Fertil Steril*, **35**: 105-123

Schipper, I., de Jong, F. H., and Fauser, B. C. (1998) Lack of correlation between maximum early follicular phase serum follicle stimulating hormone concentrations and menstrual cycle characteristics in women under the age of 35 years. *Hum Reprod*, **13**: 1442-1448

Schoemaker, J., van Weissenbruch, M. M., Scheele, F., and van der Meer, M. (1993) The FSH threshold concept in clinical ovulation induction. *Baillieres Clin Obstet Gynaecol*, **7**: 297-308

Schoot, D. C. and Fauser, B. C. J. M. (1992) Monitoring of follicular development during induction of ovulation using exogenous gonadotrophins. *Exp.Clin.Endocrinol*, **11**: 303-306

Schoot, D. C., Pache, T. D., Hop, W. C., de Jong, F. H., and Fauser, B. C. (1992) Growth patterns of ovarian follicles during induction of ovulation with decreasing doses of human menopausal gonadotropin following presumed selection in polycystic ovary syndrome. *Fertil Steril*, **57**: 1117-1120

Schoot, D. C., Hop, W. C., de Jong, F. H., van Dessel, T. J., and Fauser, B. C. (1995) Initial estradiol response predicts outcome of exogenous gonadotropins using a step-down dose regimen for induction of ovulation in polycystic ovary syndrome. *Fertil Steril*, **64**: 1081-1087

Schoot, D. C., Hop, W. C., Pache, T. D., de Jong, F. H., and Fauser, B. C. (1993) Growth of the dominant follicle is similar to normal in patients with gonadotrophin-stimulated polycystic ovary syndrome exhibiting monofollicular development during a decremental dose regimen. *Acta Endocrinologica*, **129**: 126-129

Schwartz, M., Jewelewicz, R., Dyrenfurth, I., Tropper, P., and Vande, Wiele (1980) The use of human menopausal and chorionic gonadotropins for induction of ovulation. Sixteen years' experience at the Sloane Hospital for Women. *Am J Obstet Gynecol*, **138**: 801-807

Schwartz, M. and Jewelewicz, R. (1981) The use of gonadotropins for induction of ovulation. *Fertil Steril*, **35**: 3-12

Scott, R. T., Opsahl, M. S., Leonardi, M. R., Neall, G. S., Illions, E. H., and Navot, D. (1995) Life table analysis of pregnancy rates in a general infertility population relative to ovarian reserve and patient age. *Hum Reprod*, **10**: 1706-1710

Seibel, M. M., Kamrava, M. M., McArdle, C., and Taymor, M. L. (1984) Treatment of polycystic ovary disease with chronic low-dose follicle stimulating hormone: biochemical changes and ultrasound correlation. *Int J Fertil*, **29**: 39-43

Shepard, M. K., Balmaceda, J. P., and Leija, C. G. (1979) Relationship of weight to successful induction of ovulation with clomiphene citrate. *Fertil Steril*, **32**: 641-645

Shoham, Z., Borenstein, R., Lunenfeld, B., and Pariente, C. (1990) Hormonal profiles following clomiphene citrate therapy in conception and nonconception cycles. *Clinical Endocrinology*, **33**: 271-278

Shoham, Z., Patel, A., and Jacobs, H. S. (1991a) Polycystic ovarian syndrome: safety and effectiveness of stepwise and low-dose administration of purified follicle-stimulating hormone. *Fertil Steril*, **55**: 1051-1056

Shoham, Z., Zosmer, A., and Insler, V. (1991b) Early miscarriage and fetal malformations after induction of ovulation (by clomiphene citrate and/or human menotropins), in vitro fertilization, and gamete intrafallopian transfer . *Fertil Steril*, **55**: 1-11

Snick, H. K., Snick, T. S., Evers, J. L., and Collins, J. A. (1997) The spontaneous pregnancy prognosis in untreated subfertile couples: the Walcheren primary care study. *Hum Reprod*, **12**: 1582-1588

Stein, I. F. and Leventhal, M. L. (1935) Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol*, **29**: 181-191

Stein, I. F. (1967) Duration of fertility after wedge resection of the ovary in Stein-Leventhal syndrome. *Gynecol Prat*, **18**: 9-19

Strowitzki, T., Seehaus, D., Korell, M., and Hepp, H. (1994) Low-dose follicle stimulating hormone for ovulation induction in polycystic ovary syndrome. *J Reprod Med*, **39**: 499-503

Swanson, M., Sauerbrei, E. E., and Cooperberg, P. L. (1981) Medical implications of ultrasonically detected polycystic ovaries. *J Clin Ultrasound*, **9**: 219-222

Taymor, M. L., Sturgis, S. H., Goldstein, D. P., and Lieberman, B. (1967) Induction of ovulation with human postmenopausal gonadotropin. 3. Effect of varying dosage schedules on estrogen and pregnanediol excretion levels. *Fertil Steril*, **18**: 181-190

Templeton, A., Morris, J. K., and Parslow, W. (1996) Factors that affect outcome of in-vitro fertilisation treatment. *Lancet*, **348**: 1402-1406

The ESHRE Capri Workshop Group. (1995) Anovulatory infertility. *Hum Reprod*, **10**: 1549-1553

Thompson, C. R. and Hansen, L. M. (1970) Pergonal (menotropins): a summary of clinical experience in the induction of ovulation and pregnancy. *Fertil Steril*, **21**: 844-853

Tietze, C. (1968) Fertility after discontinuation of intrauterine and oral contraception. *Int J Fertil*, **13:** 385-389

Tiitinen, A. E., Laatikainen, T. J., and Seppala, M. T. (1993) Serum levels of insulin-like growth factor binding protein-1 and ovulatory responses to clomiphene citrate in women with polycystic ovarian disease. *Fertil Steril*, **60:** 58-62

Ulloa-Aguirre, A., Midgley, A. R., Jr., Beitins, I. Z., and Padmanabhan, V. (1995) Follicle-stimulating isohormones: characterization and physiological relevance. *Endocrine Reviews*, **16:** 765-787

van Dessel, H. J., Chandrasekher, Y., Yap, O. W., Lee, P. D., Hintz, R. L., Faessen, G. H., Braat, D. D., Fauser, B. C., and Giudice, L. C. (1996) Serum and follicular fluid levels of insulin-like growth factor I (IGF-I), IGF-II, and IGF-binding protein-1 and -3 during the normal menstrual cycle. *J Clin Endocrinol Metab*, **81:** 1224-1231

van Dessel, H. J., Lee, P. D., Faessen, G., Fauser, B. C., and Giudice, L. C. (1999) Elevated serum levels of free insulin-like growth factor-1 (IGF-1) in polycystic ovary syndrome. *J Clin Endocrinol Metab*, **84:** 3030-3035

van Dessel, H. J., Schoot, B. C., Schipper, I., Dahl, K. D., and Fauser, B. C. (1996) Circulating immunoreactive and bioactive follicle stimulating hormone concentrations in anovulatory infertile women and during gonadotrophin induction of ovulation using a decremental dose regimen. , **11:** 478-485

Van Houwelingen, J. C. and Le Cessie, S. (1990) Predictive value of statistical models. *Statistics in Medicine*, **9:** 1303-1325

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

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

van Santbrink, E. J. and Fauser, B. C. (1997a) 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

van Santbrink, E. J., Hop, W. C., and Fauser, B. C. (1997b) Classification of normogonadotropic infertility: polycystic ovaries diagnosed by ultrasound versus endocrine characteristics of polycystic ovary syndrome. *Fertil Steril*, **67:** 452-458

Wang, C. F. and Gemzell, C. (1980) The use of human gonadotropins for the induction of ovulation in women with polycystic ovarian disease. *Fertil Steril*, **33:** 479-486

Weaver, J. U., Holly, J. M., Kopelman, P. G., Noonan, K., Giadom, C. G., White, N., Virdee, S., and Wass, J. A. (1990) Decreased sex hormone binding globulin (SHBG) and insulin-like growth factor binding protein (IGFBP-1) in extreme obesity. *Clin Endocrinol (Oxf)*, **33:** 415-422

White, D. M., Polson, D. W., Kiddy, D., Sagle, P., Watson, H., Gilling-Smith, C., Hamilton-Fairley, D., and Franks, S. (1996) Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. *J Clin Endocrinol Metab*, **81:** 3821-3824

Wilson, E. A., Jawad, M. J., and Hayden, T. L. (1982) Rates of exponential increase of serum estradiol concentrations in normal and human menopausal gonadotropin-induced cycles. *Fertil Steril*, **37:** 46-49

World Health Organization. (1992) WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Third edition. Cambridge: Cambridge University Press, UK.

World Health Organization. (1993) WHO manual for the standardized investigation and diagnosis of the infertile couple. Cambridge: Cambridge University Press.

Wu, C. H. (1977) Plasma hormones in clomiphene citrate therapy. *Obstet Gynecol*, **49:** 443-448

Yen, S. S., Vela, P., and Rankin, J. (1970) Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. *J Clin Endocrinol Metab*, **30:** 435-442

Yen, S. S. (1980) The polycystic ovary syndrome. *Clinical Endocrinology*, **12:** 177-207

Yki-Jarvinen, H., Makimattila, S., Utriainen, T., and Rutanen, E. M. (1995) Portal insulin concentrations rather than insulin sensitivity regulate serum sex hormone-binding globulin and insulin-like growth factor binding protein 1 in vivo. *J Clin Endocrinol Metab*, **80:** 3227-3232

Zachow, R. J. and Magoffin, D. A. (1997) Direct intraovarian effects of leptin: impairment of the synergistic action of insulin-like growth factor-I on follicle-stimulating hormone-dependent estradiol-17 beta production by rat ovarian granulosa cells. *Endocrinology*, **138:** 847-850

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature*, **372:** 425-432

Summary

Chapter 1: The first chapter of the present thesis has focused on classification of anovulatory infertility, the treatment strategies, ovulation induction protocols, success and complication rates reported in the literature, and an insentive for the development of a new classification of WHO 2 anovulatory infertility which in fact is the most common form of anovulation in women of reproductive age. We also briefly described the objective of the present thesis which has been the prediction of treatment outcome in these patients. Therefore, we have discussed several statistical aspects of development of a prognostic model in longitudinal follow-up cohort studies.

Chapter 2: In the first part of Chapter 2 was have focused on identification of certain criteria assessed during standardized initial screening which could predict the response to ovulation induction with clomiphene citrate (CC) in 201 patients presenting with oligomenorrhea or amenorrhea and infertility. Serum FSH levels were within the normal range (1-10 IU/L). First ovulation with CC was used as the end point. After a complete follow-up (in the case of a non-response, at least 3 treatment cycles with daily CC doses up to 150 mg), 156 patients (78%) ovulated. The free androgen index (FAI = testosterone/sex hormone-binding globulin ratio), body mass index (BMI), cycle history (oligomenorrhea vs. amenorrhea), serum androgen (testosterone and/or androstenedione) levels, and mean ovarian volume assessed by transvaginal sonography were all significantly different ($P < 0.01$) in responders from those in non-responders. FAI was chosen to be the best predictor in univariate analysis. The area under the receiver operating characteristics curve in a multivariate prediction model including FAI, BMI, cycle history, and mean ovarian volume was 0.82. These observations may add to ongoing discussion regarding etiological factors involved in ovarian dysfunction in these patients and classification of normogonadotropic anovulatory infertile women.

In the second part of this chapter we have tried to identify whether clinical, endocrine, or ultrasound characteristics assessed by standardized initial screening of normogonadotropic oligo/amenorrheic infertile patients could predict conception in 160 women who reached ovulation after clomiphene citrate (CC) medication. First conception (defined as a positive urinary pregnancy test) was the end point for this study. A cumulative conception rate of 73% was reached within 9 CC-induced ovulatory cycles. Patients who did conceive presented more frequently with lower age ($P < 0.0001$) and amenorrhea ($P < 0.05$) upon initial screening. In a univariate analysis, patients with elevated initial serum LH concentrations (>7.0 IU/L) had a higher probability of conceiving ($P < 0.01$). In a multivariate

analysis, age and cycle history (oligomenorrhea vs. amenorrhea) were identified as the only significant parameters for prediction of conception. These observations suggest that there is more to be gained from CC ovulation induction by younger women presenting with profound oligomenorrhea or amenorrhea. Screening characteristics involved in the prediction of ovulation after CC medication in normogonadotropic oligo/amenorrheic patients (body weight and hyperandrogenemia, as shown previously) are distinctly different from predictors of conception in ovulatory CC patients (age and the severity of cycle abnormality). This disparity suggests that ovarian response and oocyte quality (chances for conception in ovulatory cycles) may be differentially regulated.

The third part of the present prospective longitudinal follow-up study was designed to identify whether additional endocrine screening characteristics, all potentially involved in ovarian dysfunction in 182 normogonadotropic oligoamenorrheic infertile women, are associated with ovarian response, which may improve overall prediction of CC-resistant anovulation. Screening included serum assays for fasting insulin and glucose, insulin-like growth factor I (IGF-I), IGF-binding protein-1 (IGFBP-1), IGFBP-3, free IGF-I, inhibin B, leptin, and vascular endothelial growth factor. Forty-two women (22% of the total group) did not ovulate at the end of follow-up (a total number of 325 cycles were analyzed). Fasting serum insulin, insulin/glucose ratio, IGFBP-1, and leptin were all significantly different in univariate analyses ($P \leq 0.02$), comparing CC responders *vs.* nonresponders. Forward stepwise multivariate analyses in combination with factors reported earlier for prediction of patients remaining anovulatory after CC revealed a prediction model including 1) free androgen index (FAI = testosterone/sex hormone-binding globulin ratio), 2) cycle history (oligomenorrhea or amenorrhea), 3) leptin level, and 4) mean ovarian volume. These data suggest that decreased insulin sensitivity, hyperandrogenemia, and obesity - all associated with polycystic ovary syndrome - are prominent factors involved in ovarian dysfunction, preventing these ovaries from responding to stimulation by raised endogenous FSH levels due to CC medication. By using leptin instead of body mass index or waist to hip ratio, the previous model for prediction of patients remaining anovulatory after CC medication could be slightly improved (area under the curve from 0.82–0.85). This may indicate that leptin is more directly involved in ovarian dysfunction in these patients. The capability of insulin and IGFBP-1 to predict patients who remain anovulatory after CC disappears when FAI enters into the model due to a significant correlation between FAI and these endocrine parameters. This suggests that markers for insulin sensitivity (*e.g.* IGFBP-1 and insulin) are associated with abnormal ovarian function through its correlation with androgens, whereas leptin is directly involved in ovarian dysfunction.

The objective of the last part of chapter 2 is to establish whether initial screening characteristics of normogonadotropic anovulatory infertile women predict live birth after clomiphene citrate (CC) induction of ovulation. In this prospective longitudinal single centre study included 259 couples. Main outcome was conception leading to live birth after CC. Ninety eight (38%) patients conceived after CC leading to live birth. The cumulative live birth rate within 12 months was 42% for the total study population and 56% for the ovulatory women after CC. Factors predicting chances for live birth include; free androgen index (testosterone sex hormone-binding globulin ratio), body mass index, cycle history (oligomenorrhea versus amenorrhea) and woman's age. In conclusion, it is possible to predict the individual chances of live birth after CC using two distinct prediction models combined in a nomogram. Applying this nomogram in the clinic may be a step forward in optimizing the decision-making process in the treatment of normogonadotropic anovulatory infertility. Alternative first line treatment options could be considered for some women with limited chances for success.

Chapter 3: The objective of the third and last chapter of the present thesis has been prediction of the gonadotropin induction of ovulation outcome. In the first part of the present prospective longitudinal clinical study we focused on prediction of FSH response (threshold) dose in normogonadotropic, anovulatory infertile women undergoing gonadotropin induction of ovulation. Ninety clomiphene citrate (CC) resistant (CRA) or CC-failure (CCF) normogonadotropic oligoamenorrheic infertile patients were included. A low-dose step-up regimen for daily exogenous FSH administration was applied. Main outcome measures has been the FSH dose on the day of ovarian response (follicle growth beyond 10 mm diameter). The following equation has been developed applying a multivariate analyses to predict the individual FSH response dose (between 75 and > 187 IU/d) before initiation of therapy: [4 body mass index (BMI; kg /m2)] + [32 CRA (yes = 1 or no = 0)] + [7 initial free insulin-like growth factor-I (free IGF-I; ng/mL)] + [6 initial serum FSH (IU/L)] – 51. Standard error of the predicted dose = 35 IU. In conclusion, the individual FSH response dose for gonadotropin induction of ovulation in anovulatory infertile women can be predicted on the basis of initial screening characteristics. The prediction model developed in this study may increase safety and efficiency of low-dose gonadotropin protocols (both step-up or step-down) by the proper assessment of the appropriate starting dose for a given patient.

The objective of the second part of the present chapter, has been to evaluate whether standardised initial screening may predict treatment outcome (mono vs. multi-follicular development and conception) following gonadotropin induction of ovulation. One hundred and fifty two women who previously failed to ovulate or

conceive following clomiphene citrate (CC) induction of ovulation were included. The main outcome parameters included number of developing follicles assessed by ultrasound, cycle cancellations and conception (positive urinary pregnancy test) after exogenous gonadotropins. In an univariate logistic regression analyses to predict the probability of multiple dominant (> 15 mm) follicle development (n = 198, 45%), ovarian response to the preceding CC treatment, initial serum LH, FSH, testosterone (T), androstenedione (AD), and sonographic appearance of polycystic ovaries were significant different. A multivariate model to predict the chances of generating multiple dominant follicles could be developed with an area under the receiver operating characteristic curve (AUC) of 0.6, including T and mean follicle number. A total number of 79 patients conceived (52%) and 69 women had a live birth (45%). A cumulative conception rate (CCR) of 60% was reached within 6 induced cycles. In univariate Cox regression analysis patients age and initial serum insulin-like growth factor-I (IGF-I) were significant different ($P < 0.05$). Comparing those women who did versus those who did not conceive in a multivariate Cox regression analyses women's age, initial serum IGF-I and T entered into the final model, which had an AUC of 0.69. In conclusion, it is possible to predict the individual treatment outcome - chances for a successful stimulation of monofollicular growth and conception - following gonadotropin induction of ovulation applying a step-down dose regimen. The present two prediction models (in combination with the previous model predicting the individual FSH response dose) may render gonadotropin ovulation induction more patient tailored by reducing chances for complications and optimizing chances of achieving a singleton pregnancy.

Samenvatting

Hoofdstuk 1: Het eerste hoofdstuk van dit proefschrift is gericht op de klassificatie van anovulatoire infertiliteit, verschillende behandelingsstrategieen en ovulatie inductie protocollen en successen en complicaties zoals beschreven in de literatuur. Het doel van de 6 studies beschreven in dit proefschrift – predictie van uitkomsten van ovulatie inductie bij voruwen met normogonadotrope, anovulatoire infertiliteit – werd aangegeven. Statistiche aspecten van de ontwikkeling van predictie modellen in longitudinale follow-up studies worden beschreven.

Hoofdstuk 2: In het eerste deel van hoofdstuk 2 lag de focus op identificatie van bepaalde criteria (verkregen tijdens een gestandaardiseerde initiële screening) die voorspellend zouden kunnen zijn voor de respons op ovulatie inductie met clomifeen citraat (CC) bij 201 patiënten met oligomenorroe of amenorroe en infertiliteit. Serum FSH spiegels waren binnen de normale range (1-10 IU/l). De eerste ovulatie met CC werd gebruikt als eindpunt. Na een complete follow-up (in geval van geen respons, tenminste 3 behandelingscycli met een dagelijkse CC dosering tot 150 mg) waren 156 patiënten (78%) ovulatoir. De vrije androgeen index (FAI = free androgen index = ratio testosteron/sexhormoon bindend globuline), BMI (body mass index), cyclus anamnese (oligomenorroe versus amenorroe), serum androgeen spiegels (testosteron en/of androsteendion), en het gemiddelde ovarium volume (bepaald met behulp van transvaginale echografie) waren allen significant verschillend ($P < 0.01$) tussen patiënten met en zonder respons op CC. De FAI werd gekozen als de beste voorspeller voor ovariële respons in de univariate analyse. De "area onder de ROC (receiver operating characteristics) curve" in een multivariaat predictie-model met FAI, BMI, cyclus anamnese, en gemiddeld ovarium volume was 0.82. Deze bevindingen zijn wellicht van belang voor de voortdurende discussie over etiologische factoren betrokken bij ovarium dysfunctie bij deze patiënten en voor de classificatie van normogonadotrope anovulatoire infertiele vrouwen.

In het tweede deel van het hoofdstuk hebben we geprobeerd om klinische, endocriene of echo karakteristieken (bepaald bij gestandaardiseerde initiële screening van normogonadotrope infertiele patiënten met oligo/amenorroe) te identificeren die voorspellend zijn voor conceptie bij 160 patiënten die ovuleerden na CC medicatie. De eerste conceptie (gedefinieerd als een positieve urine zwangerschapstest) was het eindpunt van deze studie. De cumulatieve zwangerschapskans van 73% werd bereikt binnen 9 CC-geïnduceerde ovulatoire cycli. De patiënten die zwanger werden, hadden vaker een jongere leeftijd ($P < 0.0001$) en amenorroe ($P < 0.05$) bij de initiële screening. In de univariate analyse hadden patiënten met een

verhoogde initiële LH serum spiegel (> 7.0 IU/l) een grotere kans op zwangerschap ($P < 0.01$). In de multivariate analyse werden leeftijd en cyclusanamnese (oligo- of amenorroe) geïdentificeerd als de enige significante parameters voor de predictie van conceptie. Deze bevindingen suggereren dat er bij ovulatie inductie met CC meer winst valt te behalen bij jongere vrouwen met ernstige oligomenorroe of amenorroe. De screenings karakteristieken die betrokken zijn bij de predictie van ovulatie na CC medicatie bij normogonadotrope patiënten met oligo/amenorroe (BMI, en hyperandogenemie, zie eerder) zijn duidelijk verschillend van de voorspellers van conceptie in ovulatoire CC patiënten (leeftijd en mate van cyclusstoornis). Dit onderscheid suggereert dat de FSH drempel (hoeveelheid FSH nodig voor stimulatie van follikelgroei en ovulatie) en oocyt kwaliteit (kans op conceptie in ovulatoire cycli) verschillend worden gereguleerd.

Het derde deel van de huidige prospectieve longitudinale follow-up studie was ontworpen om te beoordelen of additionele endocriene screenings parameters, allen potentieel betrokken bij ovariële dysfunctie in 182 normogonadotrope infertiele vrouwen met oligo/amenorroe, correleerden met ovariële respons, zodat wellicht de totale predictie van CC-resistente anovulatie kan worden verbeterd. Bij de screening waren analyses voor nuchtere insuline en glucose, IGF-I (insulin-like growth factor I), IGFBP-1 (IGF-binding protein-1), IGFBP-3, vrij IGF-I, inhibine B, leptine, en "vascular endothelial growth factor" inbegrepen. Twee-enveertig vrouwen (22% van de totale groep) ovuleerden niet aan het eind van de follow-up (in totaal werden 325 cycli geanalyseerd). Het nuchtere serum insuline, de insuline/glucose ratio, IGFBP-1, en leptine waren allen significant verschillend in univariate analyses ($P \leq 0.02$) tussen de patiënten met en zonder ovariële respons. Met "forward stepwise" multivariate analyses, in combinatie met de factoren die eerder zijn aangegeven voor de predictie van patiënten die anovulatoir blijven na CC, kwam een predictiemodel tot stand met 1) FAI, 2) cyclusanamnese, 3) leptine serum spiegel, en 4) gemiddeld ovarium volume. Deze data suggereren dat een verlaagde insuline sensitiviteit, hyperandrogenemie, en obesitas (allen geassocieerd met polycysteus ovarium syndroom), prominente factoren zijn betrokken bij ovariële dysfunctie, en voorkomen dat de ovaria van deze patiënten reageren op stimulatie door verhoogde endogene FSH spiegels ten gevolge van CC medicatie. Door leptine te gebruiken in plaats van BMI of taille/heup ratio, kon het eerdere model voor predictie van patiënten die anovulatoir bleven na CC medicatie enigszins verbeterd worden ("area under the curve" van 0.82 naar 0.85). Dit kan betekenen dat leptine directer betrokken is bij ovarium dysfunctie bij deze patiënten. De competentie van insuline en IGFBP-1 om patiënten te voorspellen die anovulatoir blijven na CC verdwijnt zodra FAI het model binnenkomt ten gevolge van een significante correlatie tussen FAI en deze endocriene parameters. Dit suggereert dat markers

voor insuline sensitiviteit (zoals IGFBP-1 en insuline) geassocieerd zijn met abnormale ovarium functie door hun correlatie met androgenen, terwijl leptine direct betrokken is bij ovarium dysfunctie.

Het doel van het laatste deel van hoofdstuk 2 is om vast te stellen of initiële screenings karakteristieken van normogonadotrope anovulatoire infertiele patiënten voorspellend zijn voor de geboorte van een levend kind na de inductie van ovulatie met CC. In deze prospectieve longitudinale studie werden 259 paren bestudeerd met een voorgeschiedenis van infertiliteit, oligo/amenorroe, normale FSH (follikel stimulerend hormoon) concentraties en geen eerdere behandeling met enige medicatie voor ovulatie inductie. De belangrijkste uitkomst was conceptie na CC leidend tot de geboorte van een levend kind. Negenentachtig (38%) patiënten werden zwanger na CC leidend tot de geboorte van een levend kind. Het cumulatieve percentage levende geboortes binnen 12 maanden was 42% voor de totale studie-populatie en 56% voor de ovulatoire vrouwen na CC. De factoren die de kans op de geboorte van een levend kind voorspellen zijn: FAI, BMI, cyclusanamnese (oligo- of amenorroe) en de leeftijd van de vrouw. Concluderend, het is mogelijk om de individuele kansen voor de geboorte van een levend kind na CC gebruik te voorspellen in twee verschillende predictie modellen, gecombineerd in een nomogram. De toepassing van dit nomogram in de kliniek kan een stap vooruit zijn in het verbeteren van het besluitvormingsproces in de behandeling van normogonadotrope anovulatoire infertiliteit. Voor sommige vrouwen met lage kansen op succes kunnen alternatieve eerstelijns behandelings opties worden overwogen.

Hoofdstuk 3: Het 3^{e} en laatste hoofdstuk van dit proefschrift behandelt het voorspellen van de uitkomst ovulatie-inductie met gonadotrofines. In het eerste deel van de huidige prospectieve longitudinale studie hebben we ons gericht op voorspellen van de FSH response-(drempel) dosis in normogonadotrope anovulatoire infertiele vrouwen die gonadotrofine-ovulatie inductie ondergingen. Negentig normogonadotrope anovulatoire vrouwen die clomifeencitraat (CC)- resistent (CRA) waren en CC- behandelde vrouwen die wel ovuleerde, maar niet zwanger werden (CCF), werden geïncludeerd. Voorafgaand aan de behandeling werd een gestandaardiseerde screening gedaan waarbij klinische-, endocriene en echografische parameters werden vastgelegd. Vervolgens werd in een low-dose step-up protocol dagelijks exogeen gonadotrofine toegediend. De FSH dosis op de dag van ovariële response (follikel groei > 10 mm in diameter) werd gezien als de FSH-response dosis. Met behulp van een multivariate analyse is de volgende formule ontwikkeld met als doel om de individuele FSH-responsedosis te berekenen voorafgaand aan een behandeling: [4 body mass index (BMI; kg /m2)] + [32 CRA

(ja = 1 or nee = 0)] + [7 initial vrije- insulin-like growth factor-I (free IGF-I; ng/mL)] + [6 initial serum FSH (IU/L)] – 51. Standaard error van de voorspelde dosis is 35IU/L. Concluderend kan worden gesteld dat de individuele FSH-respons dosis voor gonadotrofine ovulatie inductie in normogonadotrope anovulatoire infertiele vrouwen kan worden voorspeld op basis van screenings-parameters. Het in deze studie ontwikkelde predictie-model kan veiligheid en efficiëntie van een low-dose gonadotrophine protocol (zowel step-up als step-down) verhogen door de juiste start dosis te bepalen bij een individuele patiënt.

In het tweede deel van het huidige hoofdstuk wordt gekeken of klinische, endocriene, of echografische bevindingen tijdens de gestandaardiseerde screening voorafgaande aan de behandeling, de uitkomst van de behandeling (mono- vs multi-folliculaire groei en conceptie) kunnen voorspellen bij gonadotrofine ovulatie inductie. Honderdtweeënvijftig vrouwen werden geïncludeerd. Gekeken werd naar het aantal groeiende follikels bij echografisch onderzoek, het aantal afgebroken cycli en conceptie (positieve urinaire zwangerschapstest). Bij een univariate logistieke regressie analyse ter voorspelling van ontwikkeling van multipele dominante follikelgroei (>15 mm in diameter) bleek ovariële response op de voorafgaande CC behandeling, initieel LH, FSH, Testosteron (T), Androstendion (AD) en echografische PCO beeld significant te verschillen. Een multivariate model werd ontwikkeld om de kans voor het optreden van multipele dominante follikelgroei te voorspellen met behulp van de initiële serum Testosteron en het aantal follikel op de dag van screening. In totaal werden 79 patiënten zwanger (52%) waarvan 69 (45%) vrouwen van een levendgeboren kind bevielen. Een cumulatieve conceptie rate (CCR) van 60% werd bereikt binnen 6 behandelcycli. In een univariate Cox-regressie analyse werden leeftijd, initiele IGF-I en T toegevoegd aan het model waarbij een AUC van 0.69% kon worden bereikt. Concluderend lijkt het mogelijk de individuele behandeluitkomst – de kansen voor succesvolle monofolliculaire groei en conceptie – bij een low-dose step-down gonadotrofine ovulatie-inductie protocol te voorspellen. Deze twee predictiemodellen kunnen deze behandeling gebuikvriendelijker maken en ons helpen om de kans op complicaties tijdens de behandeling te minimaliseren en de kansen op een eenlingzwangerschap te vergroten.

Publications

Publications included in the present thesis:

Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (1998) Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, **83**: 2361-2365

Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (1999) Predictors of chances to conceive in ovulatory patients during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, **84**: 1617-1622

Imani, B., Eijkemans, M. J., de Jong, F. H., Payne, N. N., Bouchard, P., Giudice, L. C., and Fauser, B. C. (2000) Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J Clin Endocrinol Metab*, **85**: 676-682

Imani, B., Eijkemans, M. J., te Velde, E. R., Habbema , J. D., and Fauser, B. C. (2002) A nomogram to predict live birth probability after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *Fertil Steril*, **77**: 91-97

Imani, B., Eijkemans, M. J., Faessen, G., Bouchard, P., Giudice, L. C., and Fauser, B. C. (2002) Prediction of the individual follicle-stimulating hormone threshold for gonadotrophin induction of ovulation in anovulatory infertility: An approach to minimize multiple gestation, ovarian hyperstimulation, and treatment expense. *Fertil Steril*, **77**: 83-90

Publications from the author related to the present thesis:

Macklon NS, Imani, B, Fauser, BC. 1999. Optimizing protocols for ovulation induction. *In: Female Infertility Therapy, current practice*, Z. Shoham, C. M. Howles H. S. Jacobs (Eds.), Martin Dunitz. pp. 75 - 83.

Bili, H, Laven, JS, Imani, B, Eijkemans, MJ (2001). Age related changes in clinical, endocrine and sonographic ovarian characteristics in normogonadotropic oligoamenorrheic infertile women of reproductive years. *Eur J Endocrinol*, **145**: 749-55.

Laven, JS, Imani, B, Eijkemans, MJ, de Jong, FH, Fauser, BC (2001) Absent biologically relevant associations between serum inhibin B concentrations and characteristics of polycystic ovary syndrome in normogonadotrophic anovulatory infertility. *Hum Reprod*, **16**: 1359-64.

Imani, B, Eijkemans, MJC, Habbema, JDF, Te Velde, ER, Fauser, BC (2002) Ovulation induction: Predictors of ovarian response and clinical outcomes. In; *Polycystic Ovary Syndrome*. RJ Chang, A Dunaif, JJ Heindel, eds. Marcel Dekker. In Press

Giudice LC, Conover CA, Bale L, Faessen GH, Ilg K, Sun I, B, Suen LF, Irwin JC, Christiansen M, Overgaard MT, Oxvig C. (2002) Identification and regulation of the IGFB-4 protease and its physiological inhibitor in human trophoblasts and endometrial stroma: evidence for paracrine regulation of IGF-II bioavailability in the placental bed during human implantation. *J Endocrinol Metab*, **87**: 2359-66

Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A, Osteen K, Taylor Rn, Lessey BA, Giudice LC (2002) Global gene profiling in human endometrium during the window of implantation. *Endocrinology*, **143**: 2119-38

Abstracts and presentations from the author related to the this thesis:

Imani, B; Eijkemans, MJ; te Velde, ER; Habbema, JD; Fauser, BC. Predictive value of initial screening in normogonadotropic oligoanovulatory infertility during clomiphene citrate induction of ovulation. Endocrine Society for Human Reproductive Endocrinology (ESHRE), Edinburg, UK. June 1997.

Imani, B; Eijkemans, MJ; te Velde, ER; Habbema, JD; Fauser BC. Discrepancies in predictors of ovulation and conception following clomiphene citrate induction of ovulation in normogonadotropic oligo-amenorrheic infertility. 16th World Congress on Fertility and Sterility and the 54th Annual Meeting of the American Society for Reproductive Medicine, San Francisco, California, USA. October 3-9, 1998.

Imani, B; Eijkemans, MJ; Giudice, LC; Faessen G.; Bouchard, P; Fauser, BC. Predictors of individual follicle-stimulating hormone threshold level for gonadotrophin induction of ovulation in anovulatory infertility. Submitted for the 16th Annual Meeting of the Endocrine Society for Human Reproductive Endocrinology (ESHRE), Bologna, Italy. June 25-28, 2000.

Imani, B; Eijkemans, MJ; Fauser, BC. Life table analyses and prediction of chances for pregnancy during clomiphene citrate induction of ovulation in the overall group of normogonadotropic oligoamenorrheic infertility. Submitted for the 56th Annual Meeting of the American Society for Reproductive Medicine, San Diego, California, USA. October 21-25, 2000.

Curriculum vitae auctoris

De schrijver van dit proefschrift, Babak (Bâ-Back) Imani werd op 17 Augustus 1964 geboren te Teheran, Iran. Hij heeft de middelbare school afgemaakt in 1980 in "the College of Kharazmi" te Teheran, wat zonder twijfel nummer één en beste college na Alborz College is. Hij is 2 jaar later, vanwege zijn studie, met zijn zoektocht begonnen om van Het Oosten naar Het Westen te komen. Het eerste station werd Istanbul, Turkije. Hier heeft hij in 1989 de studie Geneeskunde afgemaakt. Zijn tocht is daarna verder gegaan in westelijke richting, namelijk Nederland. Hij heeft eerst Katwijk, Goes, en daarna Dordrecht gekozen als woonplaats en uiteindelijk is hij in Rotterdam beland. De studie geneeskunde heeft hij aan de Erasmus Universiteit Rotterdam afgemaakt in 1994. Vanaf 1994 tot 2001 is hij bezig geweest om de data te verzamelen van 880 patiënten die behandeld werden binnen de Sector Voortplantings geneeskunde te Rotterdam (EMCR). Dit werk heeft geleid tot de totstandkoming van zes artikelen over prediktiemodellen in de behandeling van vrouwen met ongewenste kinderloosheid op basis van onregelmatige menstruaties. Hij heeft in januari 2001 samen met Roya en Dariush zijn tocht nog iets verder voortgezet naar het Westen: naar de zon van California, en de wetenschap van Stanford University. Hij is werkzaam binnen de afdeling Obstetrie en Gynecology, sectie reproductive Endocrinology van Stanford als postdoctoraal student op het gebied van ovarium functie en endometrium receptiviteit, en de data analyses van Microarray Chip Technology. Hij is daarbij de databasemanager van het IVF laboratorium.
Hij is van plan om binnenkort in een Bio-Tech company die gevestigd is in Palo Alto en gerelateerd is aan Stanford University te gaan werken als "Senior Scientist" en "Application Specialist".
Zijn hobbies zijn, muziek, keyboards, badminton, het verzamelen van oude antieke zwart-wit foto's, boeken, computersoftware en database.

Hij denkt dat hij zijn tocht hierbij zal laten, maar de wereld is rond. Als hij zijn tocht zal voortzetten, zal hij zich in het verre Oosten of in zijn eigen land ooit terug kunnen vinden...

Dankwoord

Zonder iemand tekort te willen doen zou ik hierbij graag vele mensen hartelijk willen bedanken die een bijzondere bijdrage hebben geleverd aan de totstandkoming van dit proefschrift. Helaas vergeet ik ongetwijfeld een aantal mensen waar ik hiervoor reeds mijn excuus aanbied. Dit onderzoek had nooit uitgevoerd kunnen worden zonder de medewerking van alle patiënten (couples - echtparen) van onze polikliniek verloskunde en vrouwenziekten Ik zal mij de meeste namen en gezichten de komende jaren nog kunnen herinneren, vooral de grote blijdschap wanneer een doorgaande zwangerschap (≥ 12 weken) het resultaat was van onze behandelingen. Ik wens jullie zeer gezonde, vrolijke jaren toe met jullie gezin en gezinsuitbreiding.

Als eerste wil ik mijn "Major" promotor en "baas", professor Fauser noemen, voor al die voortdurende steun van de afgelopen jaren. Vanaf onze eerste ontmoeting (tijdens mijn keuzeonderzoek in oktober 1994 en jullie terugkomst uit de Verenigde Staten) tot een paar dagen geleden ben je altijd mijn belangrijkste motiverende factor geweest om door te zetten. Ik wil je danken voor de investering van tijd en energie die in mijn begeleiding is gaan zitten zodat ik kon worden wat ik nu ben en voor het proeven van het genot van de wetenschappelijke experimenten. Als tweede wil ik mijn copromotor professor Habbema noemen en bedanken voor de uitstekende samenwerking en uw visie in "sofisticated statistical analyses". Ook professor Te Velde wil ik hartelijk danken voor deelname aan elke discussie en opbouwende kritiek die geleid heeft tot de totstandkoming van clomid prediktieregels. Ik dank u voor deelname aan de promotiecommissie en kritische lezing van het manuscript. Professor Helmerhorst dank ik voor deelname aan de promotiecommissie. I would like to thank professor Collins for joining the committee and his critical review of the second chapter of the present thesis. I would like to thank professor Bouchard for the collaboration regarding the clomiphene studies.

I would like to thank professor Giudice in particular for giving me the chance to join her group at the Stanford University, and the already conducted and ongoing new collaborations with EMCR. Thanks for spending time on me in your laboratory and letting me become acquainted with basic science. Thanks a lot to Gerry Faessen and Kim Chi.

Graag wil ik alle vrienden, collegae niet vergeten die door hun ondersteuning, medeleven, interesse, een kritische inbreng aan alle voordrachten en de broodnodige ontspanning belangrijke bijgedragen hebben geleverd aan het tot stand komen van dit stuk. Mijn dank aan allen. Het was een mooie tijd! Ik zal niet iedereen noe-

men. Er zijn zeker vrienden die ons hebben verlaten maar wij zullen ons hen altijd blijven herinneren voor hun liefde en gezelligheid. Ik wil graag zeker twee namen noemen; Lisette en Cees. Beste Jollanda en Ruud Rijkers, Yvonne du Pree, ik zal de "Viskaarweg" nooit vergeten. Bedankt voor jullie steun en de gezelligste tijd aller tijden. De beste onbezorgde tijd blijft maar bestaan in mijn geheugen. Het lijkt alsof het nooit terug gevonden zal worden. Een speciaal woord van dank voor Evert van Santbrink, Jits Schipper, Arne van Heusden, de "drie major follicle boys" in Nederland bij wie ik altijd welkom was. Natuurlijk moet men Thierry van Dessel en Thierry Pache niet vergeten te noemen. Onze "follicle girls" wil ik ook bedanken Femke Hohmann, Annemarie de Vet voor de steun die zij mij gaven in alle tijden. Dat jullie nu mijn paranimf willen zijn maakt het nog eens extra bijzonder. Ook dank aan Diederick de Jong, Bernd Berning, Dick Schoot, Grada van den Dool, Mark van der Gaast, Nicole Beckers, en Annemarie Mulders voor de discussies omtrent de laatste wetenschappelijke bevindingen. René Eijkemans, zonder jouw inzet en geduld had ik nooit zo ver kunnen komen in de statistische analyse van de verzamelde data van de afgelopen jaren. Dank je, voor je vriendschap in je vrije tijd, je bereikbaarheid ten allen tijde, en je organisatorisch talent en geduld voor en na mijn vertrek naar de Verenigde Staten. Ik wil graag de naam van Joke Kuijpers, Steef Blok en Eveline Ikking noemen voor de steun en het geduld bij alle organisatorische zaken. Verder dank ik Nick Macklon en Joop Laven, David Vestegen, Hans Moes, Wouter Moerdijk, Wim en Anja van Nederpeld. Beste Ravin, Ali en Marina Karimi, familie Ganai, Shehshkalani, en Alizadeh, jullie hebben een speciale plaats in mijn hart. Bedankt voor jullie steun. Familie Mozafar, Gerard en Hennie Menken, Pieter Butz, bedankt voor jullie steun. Ik zal jullie niet vergeten.

Ik mocht en mag me gelukkig prijzen met de hulp van: De staf en verpleegkundigen van de polikliniek Verloskunde en Vrouwenziekten, AZR, voor de faciliteiten en het vele prikwerk. De medewerkers van het lab Interne III en CKCL: Helene, Ronald, Jolanda en Bert, voor het vriezen, dooien, sparen, en de eindeloze hormoonbepalingen.

Na al deze bedankjes wil ik mijn ouders, broers en zus bedanken voor hun onuitsprekelijke lange geduld om deze promotie af te wachten. Mijn familie, waarvan alle steun voortdurend aanwezig was, in de goede tijden en minder goede tijden. Dit geldt natuurlijk ook voor Roya en Dariush, die hun oprechte belangstelling zonder twijfel bleef en blijft bestaan. Mijn ouders, die mij hebben gesteund. Roya, Dariush you gave me the motivation and energy to move on. Thanks to both of you. Thanks to Siamak, Ramin and Shahin, Lida and Mehrdad. I appreciate your patience.