ULTRASOUND AND PLASMA 17β-OESTRADIOL IN THE FOLLICULAR PHASE - PARTICULARLY IN IVF CYCLES

ULTRAGELUIDONDERZOEK EN PLASMA 17β-OESTRADIOL-BEPALINGEN TIJDENS DE FOLLICULAIRE FASE - IN HET BIJZONDER VAN IVF-CYCLI

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF.DR. A.H.G. RINNOOY KAN
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 29 JUNI 1988 TE 15.45 UUR.

DOOR

ROBERT ALEXANDER LEERENTVELD

GEBOREN TE DEVENTER

1988
DRUKKERIJ J.H. PASMANS B.V., 's-GRAVENHAGE

PROMOTIE COMMISSIE:

PROMOTOREN:	PROF. JHR. DR. J.W. WLADIMIROFF PROF. DR. G.H. ZEILMAKER
OVERIGE LEDEN:	PROF. DR. A.C. DROGENDIJK PROF. DR. G. HENNEMANN

To Alexander
Diederik
Rogier

CONTENTS

LIST OF ABBREVIATIONS

AID	Artificial insemination with donor semen
BBT	Basal body temperature
CC	Clomiphene citrate
E2	17β-oestradiol
ET	Embryo transfer
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin releasing hormone
HCG	Human chorionic gonadotrophin
HMG	Human menopausal gonadotrophin
HPG	Human pituitary gonadotrophin
IVF	In-vitro fertilization
LH	Luteinizing hormone
LUF	Luteinized unruptured follicle
MFD	Mean follicular diameter
MHz	MegaHertz
NS	Not significant
P	Progesterone
PCT	Post coital test
PMSG	Pregnant mare serum gonadotrophins
SD	Standard deviation
SEM	Standard error of the mean
WHO	World Health Organization

CHAPTER 1

GENERAL INTRODUCTION

Although ultrasonographic observation of the female genital tract was not applied at the first successful attempt on IVF (Steptoe and Edwards, 1978), at present the majority of IVF centres employ ultrasonographic observation of folliculogenesis as a major monitoring method during ovarian hyperstimulation therapy for IVF. The introduction in the early eighties of real-time sector scanners with high resolution, allowed rapid and reliable identification and measurements of growing Graafian follicles. The development of very small sector probes which can be introduced in the vagina not only provided improved accuracy of follicle measurements, but also meant a major breakthrough in ultrasonographically guided puncturing techniques.

Another monitoring method is determination of plasma or urinary oestrogens, which is often used in combination with ultrasonography. Circulating oestrogens reflect the functional status of developing follicles. However, the correlation between E2 levels or E2 profiles and treatment outcome remains unclear.

Plasma or urinary LH levels are determined in many centres to detect premature LH activity and the LH surge. Other, less frequently used monitoring methods comprise cervical mucus scoring or the cornification index in the vaginal cytology as parameters of peripheral E2 levels and plasma P determinations.

1.1 Ultrasound and infertility

1.1.1 *Ultrasonography of follicular growth in spontaneous menstrual cycles*

In 1963 Donald described the full bladder technique in transvesical ultrasonography with the objective to improve the diagnosis of abdominal swellings. This technique enabled Kratochwil et al. (1972, 1973) to visualize the ovaries. Kratochwil et al. (1972) and Scheer and Goldstein (1973) were the first to describe the ultrasonographic images of intraovarian structures as Graafian follicles and theca lutein cysts. Estimation of ovarian size under normal and pathological circumstances was reported by Kratochwil et al. (1972) and Zemlyn (1975).

Improved resolution of static scanners allowed Hackelöer et al. (1977) to observe follicular growth in induced menstrual cycles. Soon afterwards the ultrasonographic characteristics of the spontaneous menstrual cycle were described by various investigators (Hackelöer and Robinson, 1978; Hall et al., 1979; Robertson et al., 1979; Hackelöer and Nitschke-Dabelstein, 1980; Renaud et al., 1980; de Crespigny et al., 1981a; Kerin et al., 1981; Wetzels and Hoogland, 1982; Smith et al., 1983; Wetzels, 1983).

At that time the resolution of real-time ultrasound equipment was generally considered insufficient for follicular growth studies (Hackelöer, 1982). Nevertheless, reports on this subject did appear, employing both real-time sector scanners (Queenan et al, 1980; Fleischer et al., 1981; Hill et al., 1982) and linear-array scanners (O'Herlihy et al., 1980a; Liukkonen et al., 1984).

Nowadays, the resolution of sector scanners has improved substantially, enabling rapid orientation in the pelvis combined with accurate measurement of follicular size, as will be discussed in Chapter 2. Linear-array scanners have now been largely abandoned as visualization of the genital tract generally is inferior when compared with mechanical sector scanners.

In the late seventies ultrasound equipment did not allow identification of Graafian follicles below a mean diameter of 10 mm (Hackelöer and Robinson, 1978; Hackelöer et al., 1979; O'Herlihy et al., 1980a). At present growing follicles can be visualized from a mean diameter of 5 mm onwards.

The increase of the MFD during the follicular phase seems to be linear (Hackelöer et al., 1979; Robertson et al., 1979; O'Herlihy et al., 1980a; Queenan et al., 1980; Freundl et al., 1981), at least up to the LH surge. The daily increase in MFD is 1.5-3 mm (Hackelöer and Robinson, 1978; O'Herlihy et al., 1980a; Renaud et al., 1980; Wetzels, 1983), although incidentally a larger range has been established (1-4 mm /day; (Ylöstalo et al., 1979)). Findings on the behaviour of the follicle during and following the LH surge are conflicting, probably as a result of differences in definition of the LH surge and ultrasonographic signs of ovulation. Hackelöer et al. (1979) described a constant or even decreasing follicular size immediately prior to ovulation, whereas Queenan et al. (1980) established an exponential growth of both follicular diameter and calculated follicular volume. Renaud et al. (1980) and Wetzels (1983) observed a linear growth up to the moment of follicle disappearance.

Table 1.1
Maximum preovulatory follicular dimensions in spontaneous cycles as reported in the literature

	Follicular diameter (mm)
Hackelöer and Robinson (1978)	18 – 24
Robertson et al. (1979)	$\leq$ 25
Ylöstalo et al. (1979)	12.8
O'Herlihy et al. (1980a)	17 – 24
Renaud et al. (1980)	27
Smith et al. (1980)	25.5 $\pm$ 1.0(SEM)
Queenan et al. (1980)	21.1 $\pm$ 3.5(SD)

A wide range of maximum preovulatory follicular dimensions has been reported, as shown in Table 1.1. In the majority of studies the mean of two or three dimensions in perpendicular planes has been presented with values ranging between 18 and 25.5 mm (Hackelöer and Robinson, 1978; Robertson et al., 1979; O'Herlihy et al., 1980a; Smith et al., 1980; Queenan et al., 1980). Ylöstalo et al. (1979) measured a mean preovulatory diameter of only 12.8 mm, whereas Renaud et al. (1980) only determining the largest follicular diameter, reported values as high as 27 mm. Apart from these methodological differences, variations in individual follicular growth rate (Wetzels, 1983) and the use of different ultrasound emission velocities (1,540 or 1,600 m /sec) may have accounted for these variable findings.

1.1.2 *Ultrasonography of follicular growth in induced menstrual cycles*

As already mentioned in Section 1.1.1, the first observations on growing follicles were obtained by Hackelöer and coworkers in gonadotrophin induced cycles (1977). The enormous potential of this new technique in the surveillance of ovulation induction was rapidly recognized and a number of studies on the subject was published in a rather short period of time (Hackelöer et al., 1977; Hackelöer, 1978; Macler et al., 1978; Ylöstalo et al., 1979, 1981; Nitschke-Dabelstein et al., 1980a, 1980b; Schmidt et al., 1981; O'Herlihy et al., 1982a, 1982b; Sallam et al., 1982; Smith et al., 1983; Biggs et al., 1984).

Also here, all studies were initially performed using static B-compound scanners. Later, follicle measurements in stimulated cycles were mainly performed using high resolution mechanical sector scanners.

Several studies describe follicular growth patterns in antioestrogen or gonadotrophin induced cycles. Follicles in CC induced cycles are similar in ultrasonographic appearance and follicular size at ovulation to those developing in spontaneous cycles (Smith et al., 1980, 1983), although their growth rate is more rapid (O'Herlihy et al., 1982a). Wetzels (1983), however, only observed an accelerated growth of the dominant follicle following CC administration in the presence of multiple follicles. This is at variance with the observation of Leerentveld et al. (1985), who found a significantly faster growth of the dominant follicle in CC stimulated cycles, both under monofollicular and multifollicular conditions during stimulation for IVF. Finally, in CC induced cycles Ylöstalo et al. (1979) found significantly larger follicles at the time of ovulation when compared with spontaneous cycles. Differences in patient selection and different treatment regimens may be responsible for these conflicting findings.

Although follicular growth rates in gonadotropin induced cycles seems to vary considerably (Cabau and Bessis, 1981; O'Herlihy et al., 1982b), this does not seem to result in increased follicular dimensions at ovulation. Schmidt and coworkers (1981) reported a linear increase in the mean diameter of the dominant follicle also in HMG stimulated cycles.

Ultrasonographic observation of folliculogenesis allows accurate determination of the size and number of growing follicles in induced menstrual cycles. In the presence of multiple preovulatory follicles, HCG administration may be withheld, thus preventing multiple pregnancies. A mean follicular diameter of 18-24 mm is considered suitable for HCG administration.

Although the size, number and localization of growing preovulatory follicles resulting from ovulation induction can be easily established by means of ultrasound, it was rapidly recognized that the ovarian hyperstimulation syndrome (Engel et al., 1972) following the administration of HCG could not always be prevented by ultrasonographic surveillance (Bryce et al., 1981; Haning et al., 1983). Combined interpretation of ultrasonographic findings with urinary or plasma oestrogen levels, was therefore recommended (Seibel et al., 1981; Haning et al., 1983; McArdle et al., 1983). However, Sallam et al. (1982) and recently Bordt et al. (1986) reported that ultrasound alone can be considered reliable in ovulation induction, since the incidence of both multiple gestation and the hyperstimulation syndrome was very low. Generally the numbers of patients in these studies were too small to allow definite conclusions. Different definitions of ovarian hyperstimulation, differences in patient selection and in treatment strategy, can all account for these apparently conflicting reports.

Conclusions regarding hypostimulation or hyperstimulation of the ovaries during gonadotrophin therapy cannot be drawn from E2 measurements alone, since the size and number of developing follicles remain unknown (Schmidt et al., 1981).

1.1.3 *The role of ultrasound in ovulation prediction*

Soon after the initial descriptions of the ultrasonographical aspects of follicular growth and ovulation in spontaneous and induced menstrual cycles (Sections 1.1.1 and 1.1.2), the ability of ultrasound to predict ovulation was assessed. Despite variable follicular growth rates and maximum preovulatory dimensions, Hamilton et al. (1987a) recently found that the power of a single ultrasonographic follicle measurement to predict the moment of ovulation was superior to that based on the previous cycle length, according to McIntosh et al. (1980); in the presence of a follicle with a diameter exceeding 14 mm, ovulation took place 2¼ to 5¾ days later. Moreover, a larger number of measurements per cycle did not improve the predictive value of a single ultrasonographic follicle measurement.

Studies on the predictive value of intrafollicular echoes for the time of ovulation are conflicting. Hackelöer and Robinson (1978) described an echodense structure on the inner side of the follicle wall, in their opinion representing the cumulus oophorus ("cumulus sign"). This structure could be visualized in 80 % of the cycles and only during the 24 h period preceding ovulation. On the contrary, Kerin et al. (1981) and Wetzels (1983) found this structure in only 23 % and 15 % of the cycles respectively. Moreover, Wetzels (1983) observed the cumulus

sign during a period up to five days preceding ovulation. Picker et al. (1983) described two consistent ultrasound signs prior to ovulation: (i) a line of decreased reflectivity around the follicle appearing within 24 h of ovulation and progressing in (ii) crenation within the lining of the follicle, appearing within 6-10 h of the time of ovulation.

The use of ultrasound for timing artificial insemination was first described by Rönnberg et al. (1978). Ultrasonographic timing of AID enabled Kerin (1979) and Marinho et al. (1982) to double the pregnancy rate per cycle when compared with timing of AID based on the length of the previous cycle and BBT curves. The use of frozen donor semen requires accurate ovulation prediction, being less effective when compared with the use of fresh semen (Smith et al., 1981). Favourable results of ultrasonographic ovulation prediction for the timing of AID with frozen semen were also published by Saaranen et al. (1986).

Finally, Hamilton et al. (1986) reported that the use of ultrasound increases the prognostic value of a PCT.

1.1.4 *Ultrasonography in the diagnosis of abnormal menstrual cycles*

Following the description of the process of follicular growth, ovulation and the formation of the corpus luteum in spontaneous as well as induced menstrual cycles (Sections 1.1.1 and 1.1.2), attention was focused on ultrasonography in abnormal cycles.

In a study on follicular growth in spontaneous cycles by Renaud et al. (1980) eight out of 18 cycles had to be excluded from the study as a consequence of asynchrony of ultrasonographic findings, the BBT curve and the occurrence of E2 and LH midcycle surges. Polan et al. (1982) reported a 35 % incidence of cycles with decreased dominant follicular size, associated with abnormal serum E2 and P levels in the presence of a normal cycle length and a biphasic BBT chart. Geisthövel and coworkers (1983) correlated ultrasonographic findings with BBT curves and hormonal variables as established during normal cycles in healthy volunteers and during inadequate cycles with a short or missing luteal phase. The distinct P rise and biphasic BBT curves in some cycles without ultrasonographic evidence of corpus luteum formation suggested luteinization of regressing follicles. This finding is compatible with the LUF syndrome, as described by Jewelewicz in CC stimulated cycles (1975) and Koninckx et al. (1978) and Marik and Hulka (1978) in spontaneous cycles. The ultrasonographic appearance of these LUF cycles however is in sharp contrast with those decribed by Coulam et al. (1982), Wetzels (1983), Hamilton et al. (1985, 1987b), Janssen-Caspers et al. (1986a) and Killick and Elstein (1987), in which invariably an increase in follicular diameter following the hormonal events at midcycle was observed, often resulting in functional cyst formation. Kerin et al. (1983) defined a LUF as a follicle persisting 48 h following the LH peak; these authors did not observe the occurrence of cysts. Liukkonen et al. (1984) considered ultrasonographical absence of follicle rupture as proof of the presence of a LUF syndrome. The

incidence of the LUF syndrome as established in the above mentioned studies varies considerably (4.9-79 %), depending on patient selection, methods for confirming diagnosis (ultrasonography, laparoscopy) and the ultrasonographical definitions used.

From these studies on the ultrasonographical aspects of abnormal menstrual cycles, it may be concluded that ultrasonography provides accurate information on the morphological intra-ovarian events associated with these cycles.

1.1.5 *The use of ultrasound in in-vitro fertilization cycles*

Since the introduction of ultrasonographic follicle measurements (Hackelöer et al., 1977; Hackelöer, 1978; Hall et al., 1979; Robertson et al., 1979; Hackelöer and Nitschke-Dabelstein, 1980; Renaud et al., 1980) and their first application in ovulation induction coincided with the first successful attempts on IVF and ET (Steptoe and Edwards, 1978; Edwards et al., 1980), it was not surprising that this technique was soon applied as a monitoring tool in spontaneous and induced IVF cycles.

1.1.5.1 Ultrasound and follicular growth in in-vitro fertilization cycles

DeCrespigny and coworkers (1981b) described the use of diagnostic ultrasound in spontaneous cycles in an IVF programme. At a MFD of 17 mm, or in the presence of a 24 h urinary estrogen excretion exceeding 30 μg, the patient was admitted to hospital for urinary LH monitoring. Laparoscopic oocyte recovery was scheduled 26 to 28 h from the onset of the urinary LH surge. Laparoscopic procedures for oocyte recovery were cancelled when ultrasound demonstrated the presence of growing follicles in an ovary known to be inaccessible due to adhesions.

In 1981 Trounson et al. described the use of ultrasound in stimulated menstrual cycles; in CC stimulated cycles admittance to hospital was planned at an ultrasonographically determined follicular diameter of 17 mm. In some of their patients follicle aspiration was scheduled using the endogenous LH peak, in the remaining individuals HCG was administered 35-36 h before laparoscopy depending on follicular growth rate and the availability of the operating room. Shortly afterwards, Hoult and coworkers (1981) reported three pregnancies in CC /HCG cycles monitored by ultrasound alone. HCG was administered when the largest follicle reached a diameter of 18 mm or more. Their overall results were better in stimulated cycles when compared with spontaneous cycles.

As it gradually became clear that pregnancy rates increase with the number and quality of replaced embryos (Trounson et al., 1982; Edwards et al., 1984; Jones et al., 1984; Muasher et al., 1985; Testart et al., 1985) many IVF teams changed to induction or superstimulation treatment protocols. At present the majority of IVF teams employ a combination of ultrasonographic follicle

measurements and plasma or urinary oestrogen determinations for monitoring both morphological and functional aspects of folliculogenesis (Jones et al., 1982; Laufer et al., 1983b; Hünlich et al., 1984; McBain and Trounson, 1984; Muasher et al., 1985; Alberda et al., 1987).

1.1.5.2 Ultrasonographic appearance of the preovulatory follicle in in-vitro fertilization cycles

In 1985 Roberts et al. reported on the occurrence of intrafollicular echoes shortly before follicle aspiration. There was either a definite "cumulus sign" (Section 1.1.3) or a clear demarcation of intrafollicular echoes and the follicular wall. More oocytes and pregnancies were obtained in the patient subgroup showing intrafollicular echoes and more oocytes were mature when compared with the group with follicles without these echoes. Although these authors concluded that the presence of intrafollicular echoes may indicate follicular maturation and may be a useful predictor of a cycle likely to produce a pregnancy, these observations were not adopted in clinical practice, possibly because it was stated that adequate observation of these intrafollicular echoes required the use of a static scanner.

1.1.5.3 Ultrasonographic appearance of the endometrium in in-vitro fertilization cycles

Sakamoto and Nakano (1982) as well as Johnson et al. (1982) were the first to describe ultrasonographically detected endometrial changes during the menstrual cycle, their findings being confirmed by Hackelöer (1984a,b). Smith at al. (1984) reported four distinct patterns in the ultrasonographic appearance of the endometrium during the follicular phase. Applying these variables to their IVF program, the preoperative ovulation rate dropped from 10.9 to 3.2 % and the fertilization rate increased from 59.2 to 82.5%. The pregnancy rate per oocyte recovery and per ET, however, did not improve significantly. Glissant et al. (1985) reported on the influence of endometrial thickness on nidation prospects. IVF attempts resulting in pregnancies were associated with significantly thicker endometria; this was independent of the number of transferred embryos. However, it was not possible to predict the probability of pregnancy from endometrial thickness alone. These findings are at variance with those of Fleischer et al. (1986) and Rabinowitz et al. (1986) who could not establish differences in endometrial thickness between pregnancy and non-pregnancy cycles during the follicular phase.

Fleischer et al. (1986) reported a close relationship between endometrial thickness and plasma E2 values, both in conceptional and non-conceptional cycles. Glissant et al. (1985) and Rabinowitz et al. (1986) could not find such a correlation. These conflicting findings seem to be mainly determined by different periods

in the menstrual cycle that were included in these studies. Different stimulation regimens and differences in ultrasonographic measuring techniques may in part account for these variable results.

At present ultrasonographical observation of endometrial changes and endometrial thickness are employed incidently as an adjunct to the more commonly used monitoring techniques described in Sections 1.1.5.1 and 1.2.4.

1.1.5.4 Ultrasonically guided follicle aspiration for in-vitro fertilization

In 1981 Lenz et al. presented a technique for collecting oocytes by ultrasonically guided transvesical follicle puncture. The procedure proved to be safe and inexpensive, when compared with laparoscopic oocyte collection, allowing repeated IVF treatment (Lenz et al., 1981; Lenz and Lauritsen, 1982; Wikland et al., 1983; Lenz, 1984; Feichtinger and Kemeter, 1984; Honda et al., 1985; Trotnow et al., 1985; Janssen-Caspers et al., 1986b; Lewin et al., 1986a, 1986b). Nevertheless, the technique remained difficult and was therefore never widely accepted. Traversing the bladder is psychologically stressful for the patient. Moreover, the posterior wall of the unanaesthesized bladder must be punctured repeatedly to reach each follicle, which renders the technique rather painful. Although the number of oocytes and transplanted embryos was reduced when compared with laparoscopic oocyte retrieval, pregnancy rates were similar (Lewin et al., 1986b). This finding may be explained by the inhibition of ovarian steroidogenesis due to toxic effects of anaesthetic agents or stress induced hyperprolactinaemia as reported after general anaesthesia (Soules et al., 1980).

Following the initial report by Gleicher et al. (1983), Dellenbach and coworkers (1984, 1985) published the first series of free-hand transvaginal follicle punctures, guided by transabdominal ultrasonography. The same procedure was also successfully used by Cohen et al. (1986). Parsons et al. (1985) chose the transurethral route, also guided by transabdominal ultrasonography.

Again, technical improvements in ultrasound equipment, the development of small mechanical sector probes for vaginal application, resulted in a major change in follicle puncture techniques. Following the first report on transvaginal follicle punctures guided by transvaginal ultrasonography (Wikland et al., 1985), the first larger series were reported by Kemeter and Feichtinger (1986) and Feichtinger and Kemeter (1986), who also reported pregnancies achieved after the application of this technique. Lenz et al. (1987b) and Wikland and al. (1987) confirmed the efficacy and safety of the procedure. Previously, oocyte recovery rates at laparoscopy exceeded those obtained by ultrasonographically guided transabdominal oocyte aspirations (Janssen-Caspers et al., 1986). Presently, when employing ultrasonographically guided transvaginal follicle punctures, oocyte recovery rates equal those achieved by laparoscopy (Janssen-Caspers et al., 1988).

Although many centres use vaginal ultrasonography also for follicle measurements, to date no data on accuracy and reproducibility of this technique have been published. It is likely, however, that the accuracy of transvaginal

ultrasonographic follicle measurements will prove to be superior to that observed employing the transabdominal route as a result of the higher ultrasound frequency used with these probes and the smaller distance between the ultrasound transducer and the ovaries.

1.1.5.5 The use of ultrasound during embryo transfer

In 1985 Strickler et al. published data on the ultrasonographic surveillance of ET. Virtually no IVF centre uses ultrasound routinely for this procedure. However, the use of ultrasonically guided ET has proven valuable in patients with congenital or aquired uterine malformations.

Lenz and coworkers (1987a) reported on ultrasonographically guided transfundal ET in ten patients. Employing this technique the use of ultrasound is indispensable. From transfundal ET no pregnancies have resulted yet.

1.2 Oestrogen determinations and infertility

1.2.1 *Oestrogens in spontaneous menstrual cycles*

In the early fifties quantitative determinations of hormones (Brown, 1955) considerably extended our insight in the endocrinology of the menstrual cycle. Initially, assessment of follicular growth was made on the basis of total urinary oestrogens (Brown and Beischer, 1972). More recently, since specific, precise and sensitive radioimmunoassays became available, plasma E2 was used (Shaaban and Klopper, 1973; Black et al., 1974). Normal values for plasma E2 throughout the menstrual cycle were determined (Sanyal et al., 1974; Pepperell et al., 1975; McNatty et al., 1976) and the Graafian follicle was found to be the major source of circulating E2 (Baird and Fraser, 1974).

Except for assessment of the effectiveness of ovulation induction and ovarian stimulation relative to IVF (see Sections 1.2.2 and 1.2.4) plasma E2 determinations are now mainly used to assess the oestrogenic status in the postmenopausal patient or in the presence of climacterium praecox.

As soon as ultrasonography became available for visualisation of folliculogenesis, the correlation between oestrogen concentrations and the number and size of growing follicles was explored. A close correlation between plasma E2 levels and the dimensions of the dominant follicle in the natural cycle was found by some (Hackelöer et al., 1979; Robertson et al., 1979; Hackelöer and Nitschke-Dabelstein, 1980; Nitschke-Dabelstein et al., 1980a, 1980b; Freundl et al., 1981; Kerin et al., 1981) but refuted by others (Beck et al., 1981; Bryce et al., 1982). Probably differences in the definition of the LH surge and ultrasonographical signs of ovulation, as well as differences in statistical methodology are the main reasons for these conflicting findings.

1.2.2 *Oestrogens in induced menstrual cycles*

Ovulation induction in hypogonadotrophic women was first accomplished by Hamblen and Davis (1945), using PMSG. However, in humans, PMSG was found to be antigenic and its clinical use was completely abandoned (Buxton, 1953). Following the first report on successful induction of ovulation employing HPG (Gemzel et al., 1958) and the introduction of HMG (Lunenfeld, et al., 1962) numerous reports were published on the use of gonadotrophins for induction of ovulation. As the hazards of multiple pregnancies and overstimulation soon became clear, determination of urinary or plasma oestrogens as an index of follicular maturation was a logical consequence (Black et al., 1974; Tredway et al., 1974; Jewelewicz, 1975; Notation et al., 1978). Soon reference ranges for both urinary estrogens and plasma E2 levels were defined. Being an index of follicular function rather than of follicular growth, an important restriction of these monitoring techniques was the fact that number and size of the growing follicles remained unknown (Haning et al., 1982). Following the introduction of ultrasonographic visualisation of growing follicles, as described in Section 1.1.1, it became clear that in the past HCG had occasionally been administered too early in the treatment cycle because of the presence of multiple small follicles, resulting in a concentration of oestrogens judged suitable for induction of ovulation. Treatment became more effective employing both monitoring techniques, resulting in virtually normal conception rates (Hull, 1981; Jacobs, 1986).

In 1961 the use of CC as ovulation inducing agent was reported (Greenblatt et al.). Although the effects of CC administration on steroid production were studied extensively (Ross et al., 1970; Wu, 1977), daily treatment at present is largely based on clinical variables such as cycle length, BBT charts and midluteal plasma P determinations to confirm ovulation. Measurements of urinary or plasma oestrogens are incidentally used to confirm follicular maturation, but nowadays ultrasound plays a more important role in this respect.

Following the introduction of pulsatile GnRH in the treatment of anovulation (Leyendecker and Wildt, 1982) many groups reported on the endocrine characteristics of GnRH induced cycles (Crowley jr. et al., 1985; Abdulwahid et al., 1985; Santoro et al., 1986). Hyperstimulation and an increased incidence of multiple pregnancies has been reported (Braat et al., 1986); these risks seem however limited. At present many centres employ pulsatile GnRH therapy monitored by BBT charts, cycle length and midluteal plasma progesterone determinations to confirm ovulation.

Ylöstalo et al. (1981) and Vargyas et al. (1982) found plasma E2 levels to be closely correlated with the size and volume of the dominant follicle, the number of follicles and ovarian size. However, no such relationship could be established by others (Cabeau and Bessis, 1981; Schmidt et al., 1981; Sallam et al., 1982). Differences in patient selection are probably the main reason for these conficting findings. Amenorrheic women without endogenous oestrogenic activity (group I, WHO classification) show a strong correlation of ultrasonographic findings and E2 values during gonadotrophin stimulation. In amenorrheic

women with endogenous oestrogenic activity (group II), this correlation appears to be weak (Marrs et al., 1983; Tarlatzis et al., 1984).

1.2.3 *Oestrogens in abnormal menstrual cycles*

Determinations of urinary or plasma oestrogens in anovulatory cycles may confirm hypo-oestrogenic or hyperoestrogenic states. The use of oestrogen determinations in aberrant cycles is of limited value. Polan and coworkers (1982) found clearly abnormal serum E2 and P levels and decreased follicular size despite biphasic BBT curves and normal cycle length in some of their cycles. The correct diagnosis, however, should be made by ultrasonographic observation of follicular growth and be confirmed by plasma P determinations as indicated by Wetzels (1983).

1.2.4 *Oestrogens during the follicular phase of in-vitro fertilization cycles*

During the first successful IVF treatment cycle (Steptoe and Edwards, 1978; Edwards et al., 1980) follicular function was monitored by determinations of the 24 h urinary oestrogens. Guided by the results of these assays, 3-hourly urinary LH determinations were commenced to detect the beginning of the LH surge. Laparoscopy was performed 24-28 h later. Lopata et al. (1980) described the characteristics of the treatment cycle resulting in their first IVF pregnancy. They also used E2 measurements to decide when to admit the patient to hospital and start frequent urinary LH measurements. Ultrasonographic surveillance of follicle growth was combined with these hormonal variables.

24 h urinary oestrogen determinations also played an important role in CC or HMG induced cycles. Oestrogens served as an indicator when to start urinary LH determinations and to establish the spontaneous LH peak, whereas it was also used to pinpoint the moment of HCG administration in stimulated cycles. In most patients, a level of 75 μg/24 h was considered to be a prerequisite before HCG was given to induce the preovulatory changes in follicles.

Studies on plasma E2 levels during ovarian hyperstimulation for IVF have mainly focused on (i) E2 response in terms of absolute E2 levels (Garcia et al., 1983b; Jones et al., 1983; Dlugi et al., 1984; Laufer et al., 1986; Zarutskie et al., 1987; Forman et al., 1988), (ii) E2 profiles (Jones et al., 1983; Diamond et al., 1985; Dor et al., 1986; Laufer et al., 1986) and (iii) the duration of E2 rise above baseline values preceding follicle aspiration (Levran et al., 1985; McBain et al., 1985; Quigley et al., 1985). Pregnancy rates were highest at high preovulatory E2 levels (high responders) in several studies (Garcia et al., 1983b; Jones et al., 1983; Laufer et al., 1986). This is at variance with the findings of Forman et al. (1988), who reported decreased pregnancy rates at high preovulatory E2 levels following the transfer of one or two embryos. This difference was not observed after transfer of three embryos. Increasing E2 levels

preceding and following HCG administration (Jones et al., 1983; Diamond et al., 1985; Dor et al., 1986; Laufer et al., 1986) appeared to be associated with a higher pregnancy rate. Furthermore, a sustained E2 rise above baseline values of six days prior to the time of HCG administration was associated with a more favourable pregnancy rate when compared with that achieved after a shorter or longer period of E2 rise (Levran et al., 1985; McBain et al., 1985; Quigley et al., 1985). However, in the majority of these studies the number of treatment cycles, in particular in patient groups with an unfavourable prognosis, was too small to allow any conclusion.

In a study on the rate of plasma E2 rise (Dirnfeld et al., 1985) in the active phase of the follicular phase, the best treatment results in terms of the fertilization rate of the obtained oocytes, number of replaced embryos and finally pregnancies were achieved in cycles with a moderate "oestrogen growth rate." Both rapid and slow increases in E2 values were associated with a significantly worse treatment outcome.

Recently, Okamoto et al. (1986) published a E2 reference range derived from 102 pregnancy cycles. However, they did not compare the data of pregnancy cycles with those obtained in cycles not resulting in a pregnancy. Therefore their definition of an optimal E2 range for each cycle day may be meaningless.

Both from the studies mentioned in this paragraph and our own studies on plasma E2 levels and profiles (Sections 4.3, 4.4 and 4.5) it may be concluded that initially the predictive value of E2 determinations for IVF treatment outcome has been overestimated in smaller series. The conclusions from these preliminary studies have not been substantiated in recent larger studies. Whether the duration of E2 rise above baseline values and the rate of E2 rise in the late follicular phase have any impact on the prospects of a pregnancy has, in our opinion, yet to be determined in larger studies. At present, the importance of plasma E2 measurements for treatment policy must be reconsidered. This conclusion is supported by the recent study of Rainhorn et al. (1987), who found a similar treatment outcome in cycles with programmed oocyte recovery without any monitoring and in treatment cycles with E2 and ultrasonographic monitoring.

1.3 Luteinizing Hormone determinations and infertility

1.3.1 *Luteinizing hormone in spontaneous menstrual cycles*

Following the introduction of radioimmunoassays for gonadotrophins (Bagshawe et al., 1966; Odell et al., 1966) the secretion pattern of these glycoprotein hormones during the normal menstrual cycle could be defined (Ross et al., 1970; Hoff et al., 1983). Initially it was hypothesized that ovulation was initiated by central regulatory mechanisms (Norman et al, 1976), but in present theories the preovulatory follicle itself assumes control of its destiny by means of modulating effects on pituitary secretion of FSH and LH (Ferin et al., 1979; Knobil, 1980). Although Lemay et al. (1982) reported a considerable variation

in the duration of the LH surge and in the time interval between the initial rise of the LH surge and the moment of ovulation, the onset of the surge appears to be the most reliable indicator of impending ovulation (WHO, 1980; Testart and Frydman, 1982). Following the recent development of an enzyme-linked immunosorbent assay, various kits have become commercially available to provide a simple, self-administered and rapid test for detection of the midcycle urinary LH surge (Vermesh et al, 1987).

1.3.2 *Luteinizing hormone in induced menstrual cycles*

Ovulation induction with CC in carefully selected anovulatory patients usually results in a spontaneous LH peak (Ross et al., 1970; Wu, 1977). These peaks can be detected by repeated urinary assays and used for adequate planning of intercourse or artificial insemination. This is of great interest, especially in the presence of irregular cycles, also under CC therapy. Nowadays, patients themselves can monitor the LH surge with reliable self-administered LH kits (Vermesh et al., 1987). When folliculogenesis is present as judged by results of oestrogen assays or ultrasonography, but ovulation apparently does not occur as demonstrated by BBT charts or plasma P measurements, HCG can be administered to induce ovulation.

During ovulation induction with HMG, the LH surge usually remains absent and therefore HCG should be administered to induce ovulation. Under these circumstances urinary LH determinations do not provide any useful information, except to exclude a rare spontaneous LH surge.

Pulsatile intravenous or subcutaneous GnRH administration usually results in a "spontaneous" LH surge. In the presence of irregular, but apparently ovulatory induced cycles, urinary LH determinations can be used effectively to schedule intercourse or artificial insemination, as has been described for CC therapy (Vermesh et al., 1987).

1.3.3 *Luteinizing hormone in in-vitro fertilization cycles*

1.3.3.1 Luteinizing hormone in spontaneous in-vitro fertilization cycles

As the onset of the LH surge is the most reliable indicator of impending ovulation, it allows scheduling follicle aspiration in the spontaneous cycle (Edwards et al., 1980; Lopata et al., 1980). As a result of the pulsatile secretion pattern of GnRH and therefore of LH, the onset of the LH surge can be more accurately detected by frequent urinary LH determinations than by plasma LH measurements. The methodological difficulties in defining the LH surge have been extensively described by Testart et al. (1981).

1.3.3.2 Luteinizing hormone in stimulated in-vitro fertilization cycles

Virtually all IVF centres have now turned to stimulated cycles and plan the time of oocyte recovery by HCG administration. Initially it was reported that in normally cycling women treated with HMG for IVF purposes, the endogenous oestrogen-triggered LH midcycle surge did not occur at the expected time (Ferraretti et al., 1983; Garcia et al. 1983). Recently, however, several groups reported on the occurrence of premature LH surges in superovulation protocols for IVF (Eibschitz et al., 1986; Glasier et al., 1987; Serafini et al., 1987).

At present LH determinations in stimulated IVF cycles are used for two purposes: (i): to detect an endogenous LH surge prior to HCG administration and perform follicle puncture 24-28 h later. Many IVF teams, however, pass these cycles, since pregnancy rates following spontaneous LH surges are significantly reduced when compared with those observed after HCG administration, as reported by Lejeune et al. (1986). (ii): to assess tonic LH activity, which is of practical interest as recently has been shown by Howles and coworkers (1987) using urinary LH determinations. They found a significantly higher LH excretion in non-pregnancy cycles when compared with cycles resulting in pregnancy on days -1 and -2, day 0 being the day of HCG administration. They concluded that follicular and endocrine characteristics of pregnancy cycles and non-pregnancy cycles are indistinguishable, except for urinary LH output.

Other workers (Abdulwahid et al., 1985; Stanger and Yovich, 1985) have established a correlation between high levels of plasma LH and reduced fertilization in patients with polycystic ovaries and in patients undergoing IVF treatment. This reduced fertilization rate can be explained by: (i) resumption of meiosis at a premature stage of follicular development, resulting in aged oocytes at aspiration or (ii) altered steroid environment of the antrum sufficient to interfere with the normal development of the oocyte. Both explanations are purely hypothetical at present.

From the present data it can be concluded that premature LH activity during superovulation protocols may have a negative influence on pregnancy prospects. However, with exception of the study of Howles et al. (1987), the number of patients in the studies mentioned before are too small to allow statistically significant conclusions. As many IVF centres do not employ daily urinary or plasma LH measurements and yet have excellent treatment results, the impact of LH monitoring must be very limited.

Recently Abdalla et al. (1987) reported on the influence of the dosage of the midcycle administration of HCG on oocyte recovery rate, fertilization rate and pregnancy rate, comparing the results of injection of 2,000, 5,000, and 10,000 IU. At 2,000 IU of HCG the oocyte recovery rate was significantly lower when compared with both higher dosages. Administration of 5,000 or 10,000 IU of HCG led to similar results. Although the dosage of HCG may influence treatment outcome through different oocyte recovery rates, it may also alter the P /E2 ratio in the early luteal phase. This seems to be of practical interest as Gidley-Baird et al. (1986) found a significantly higher P /E2 ratio in women who subsequently became pregnant.

1.4 Cervical mucus changes and infertility

1.4.1 *Cervical mucus in spontaneous menstrual cycles*

Alterations in cervical mucus relative to the menstrual cycle and survival of spermatozoa in cervical mucus were first described by Sims (1868). It was Hühner (1913) who introduced analysis of cervical mucus changes as a standard part of the infertility analysis. The changes in physical properties of cervical mucus reflect follicular development and hormonal environment in an ovulatory cycle (Flynn and Bertrand, 1973). The quality of cervical mucus can be assessed employing the scoring system introduced by Insler et al. (1972). Although a high score generally is attributed to adequate follicular growth, in other hyperoestrogenic states such as polycystic ovarian disease or a persistent follicle, a favourable score can also be observed. The production of abundant cervical mucus immediately prior to ovulation can be used to plan intercourse in infertile couples.

1.4.2 *Cervical mucus in induced menstrual cycles*

Insler et al. (1972) described cervical mucus properties to be a reliable bioassay for monitoring ovulation induction. Although plasma E2 measurements and ultrasonography of ovarian follicles are at present the major monitoring techniques, cervical mucus scoring remains a simple and quick method to confirm the presence of growing follicles.

1.4.3 *Cervical mucus and in-vitro fertilization*

Garcia and coworkers (1983a) reported the use of cervical mucus changes in an IVF hyperstimulation protocol. The administration of HMG was discontinued with the appearance of the "biologic shift." This shift was defined as (i) a volume of cervical mucus > 0.2 ml; (ii) a "spinnbarkeit" >10 cm; (iii) good quality of the mucus based on its clear aspect, lack of cells and a 4 + ferning pattern. Furthermore, it was defined as 30 % pyknotic cells in vaginal cytology (Section 1.5.2) and dilatation of the external os of the cervix. HCG was administered 2-3 days afterwards depending on ultrasonographic findings.

Oelsner et al. (1986) assessed the accuracy of cervical scoring for the timing of HCG administration in HMG stimulated IVF cycles. Independent decisions to give HCG based on cervical score or a combination of E2 and ultrasound were coincident ($\pm$ 1 day) in 95 % of cases, suggesting that the cervical score can be a useful adjunct for monitoring follicular maturation in IVF patients when E2 determinations and /or ultrasonography are not available.

Matson and coworkers (1986) studied the value of the PCT in predicting the fertilization of human oocytes. There was no significant difference in the fertilization rate of oocytes whether the PCT result was negative, equivocal,

or positive; this finding was the same in both normospermic and oligospermic groups.

Evaluation of cervical mucus quantity and quality in IVF programmes is only used as a secondary method of monitoring superovulation treatment.

1.5 The karyopyknotic index in vaginal cytology and infertility

1.5.1 *The karyopyknotic index in vaginal cytology in spontaneous menstrual cycles*

It is wellknown that the karyopyknotic index in vaginal cytology reflects exposure to circulating oestrogens, as do the properties of the cervical mucus.

The only practical application of the karyopyknotic index at present is the individual determination of the oestrogenic status, as is done in postmenopausal patients and women with premature menopause.

1.5.2 *The karyopyknotic index in vaginal cytology in in-vitro fertilization cycles*

In this context, the karyopyknotic index is used in combination with cervical mucus changes and the degree of dilatation of the external cervical os, to define the "biological shift" (Garcia et al., 1983a; Section 1.4.3). The "biologic shift" generally appears in the presence of 30% pyknotic cells.

At present the karyopyknotic index is exclusively used in combination with other monitoring techniques.

1.6 The objectives our own study.

The first part of the study described in this thesis was commenced in 1982. At that time, accuracy and reproducibility of ultrasonographic follicle measurements were largely unknown. This part, therefore, has primarily been designed to assess these aspects with respect to static B-compound equipment (Section 2.2.3) and mechanical sector scanners (Section 2.2.4).

The ultrasonographical aspects of follicular growth in the spontaneous menstrual cycle have been well documented, as discussed in Section 1.1.1. Little information was available on follicular growth in stimulated cycles for IVF, which therefore comprised the second part of the study. Ovarian stimulation for IVF purposes was initially accomplished using CC. We studied follicular growth in normally cycling women using this compound both in the late follicular phase (Section 3.3.3), and in the 24 h period following the administration of HCG (Section 3.3.4). It was anticipated that the results from these studies might allow more precise planning of the administration of HCG preceding follicle aspiration.

Urinary and plasma oestrogens reflect follicular maturation both in the spontaneous and induced menstrual cycle, as described in Sections 1.2.1. and 1.2.2. The question as to whether any predictive value on the prospects of a

forthcoming pregnancy can be attributed to absolute plasma E2 levels on each treatment day or to changes in plasma E2 levels during treatment (E2 profiles) led to the third part of the study which is presented in Chapter 4. This Chapter comprises three studies on the significance of plasma E2 determinations and profiles in both CC and HMG stimulated cycles for IVF.

CHAPTER 2

ACCURACY AND REPRODUCIBILITY OF ULTRASONOGRAPHIC FOLLICLE MEASUREMENTS

2.1 Literature on accuracy and reproducibility of ultrasonographic Graafian follicle measurements.

A wide range of mean maximum preovulatory dimensions of the dominant follicle measured by ultrasound has been reported (see also Section 1.1.1.), casting serious doubt on the reliability of ultrasound equipment and measuring methods. Studies on accuracy and reproducibility of follicle measurements by ultrasound are scarce.

O'Herlihy et al. (1980b) assessed the accuracy of follicle measurements employing both static and linear-array ultrasound. A comparison was made between the ultrasonographically determined follicular volume from three maximum diameters at right angles to each other and the amount of follicular fluid obtained during laparoscopic follicle aspiration within 12 h of ultrasound examination. Although leakage of follicular fluid along the aspiration needle may have occurred, the obtained volume was used as a reference value for comparison with ultrasonographical findings. A highly significant correlation was found to exist between the MFD estimated by ultrasound and derived from follicular fluid volumes obtained at laparoscopy. The aspirated volumes tended to be smaller than those derived from the ultrasound measurement, although in nine out of 39 cases (23.1%) the ultrasound diameter was an underestimate. O'Herlihy and coworkers (1980a) also assessed the relationship between measurements on follicles using static B-compound scanning and real-time linear array equipment. A highly significant correlation was found, indicating that measurements using real-time equipment produce results similar to those obtained by the more time-consuming static scanners. Ylöstalo et al. (1981) reported similar findings, using follicle measurements in two directions.

Wetzels (1983) described calibration experiments for static B-compound scanners and real-time sector scanners. The mean relative deviation of the B-compound scanner was between 5 and 6 percent for two observers, while in his series using a sector scanner these figures amounted to 12.8 and 9.3 percent respectively. This difference between both scanning techniques was statistically significant, mechanical sector scanning being less accurate. Measuring errors in an in-vitro experiment with rubber balloons suspended in a water bath of 50-60 °C amounted to 2.6 mm in diameter.

Prins and Vogelzang (1984) also assessed reproducibility and accuracy of follicle measurements. Variability due to ultrasound equipment was clearly related

to imaging quality. A considerable interobserver variation was found with respect to the number of follicles detected. A large interobserver variation was also established regarding follicle measurements. Ultrasonographic follicle measurements, however, were only carried out in two scanning planes, thus invalidating the conclusions. Furthermore, the ultrasonographic images were recorded by sonographers, while measurements were later carried out by radiologists. Therefore, their study is not comparable with other studies on accuracy and reproducibility in which measurements were carried out in the usual interactive way.

Eissa et al. (1985) assessed reproducibility of follicle measurements using different types of modern mechanical sector scanners. Measurements were performed in three planes. The intraobserver SD was 0.6 mm and the interobserver SD 1.2 mm, irrespective of follicular diameter. The pooled SD for both variables was 1.2 mm, resulting in 95 % confidence limits of $\pm$ 2.4 mm for any measurement.

Finally, Yee and associates (1987) studied the accuracy of transvaginal follicle measurements by relating ultrasonographically determined follicular dimensions to follicular volume as obtained at laparoscopy. They established a highly significant correlation between both variables.

2.2 Introduction to own observations

The accuracy and reproducibility of follicle measurements was also established in our IVF unit in preparation of our IVF programme. The studies were performed on a static B-compound scanner (Diasonograph /EMI NE 4200, Nuclear Enterprises Ltd, Edinburgh, Scotland) with a 2.25 and 3.5 MHz internally focused transducer and a mechanical sector scanner (Combison 100, Kretztechnik GmbH, Zipf, Austria) with a 2.5 MHz transducer.

Section 2.3 comprises a study on accuracy and reproducibility of follicle measurements as determined by static B-compound MFD measurements and laparoscopically determined follicular fluid volumes.

In Section 2.4 a study on accuracy of follicle measurements performed both by static B-compound and real-time sector scanning as compared with aspirated follicular volumes at laparoscopy is presented.

2.3 Assessment of follicular development in clomiphene induced cycles by means of ultrasound and laparoscopy: a comparative study

R.A. Leerentveld, I. van Gent, A.Th. Alberda, J.W. Wladimiroff

Department of Obstetrics & Gynaecology, Erasmus University Rotterdam, The Netherlands

Published in Ultrasound in Medicine and Biology 9:595, 1983
(Reproduced with permission of the publisher, Pergamon Journals Ltd.)

Summary

Fifty CC /HCG stimulated patients undergoing laparoscopy as part of their infertility work up consented to participate in a study on: the reproducibility of ultrasonic measurement of follicular size as expressed by the inter- and intra-observer variation; the accuracy of ultrasonic assessment of location, number and size of follicles and the growth rate of the Graafian follicle during the last 12 h prior to oocyte collection. The intra- and inter-observer variation was moderate to good. Correct diagnosis of the dominant follicle was made in 73%, of the number of follicles in 72% and of follicular size (difference in size between ultrasound and laparoscopy < 2mm) in 48% of the material studied. There was a rather wide range in follicular growth rate values during the last 10-17 h prior to laparoscopy.

Introduction

Since the introduction of an ultrasound technique for imaging the ovaries and Graafian follicles (Kratochwil et al., 1972; Hackelöer et al., 1977), many reports have demonstrated its applicability in the surveillance of CC or gonadotrophin treated patients (Ylöstalo et al., 1979; Nitschke-Dabelstein et al., 1980a, 1980b; DeCherney et al., 1982; O'Herlihy et al., 1982b; Sallam et al., 1982) and its efficacy in the planning of AID (Rönnberg et al., 1978; Mastroianni et al., 1980). Lately, the technique has become an integrated part of most IVF programmes (de Crespigny et al., 1981b; DeCherney et al., 1982), allowing comparison of follicular size as measured by ultrasound and by fluid aspiration during oocyte collection. Such a programme would also open the possibility of studying follicular growth within the last 12 h prior to follicle aspiration. Little is known about the accuracy of the ultrasonic technique in the assessment of follicular size (O'Herlihy et al., 1980b; Kerin et al., 1981); no data are available on the reproducibility of this technique.

In anticipation of our own IVF programme, the purpose of the present study was to assess the reproducibility and accuracy of sonographic measurements of follicular size and also to investigate the growth of the follicle during the last 12 h prior to oocyte collection.

Material and methods

A total of 50 patients consented to participate in the proposed IVF protocol. Twelve out of these 50 patients were excluded from the study, leaving 38 patients for further evaluation. The reasons for exclusion were poor ultrasound images due to obesity and poor bladder filling, unsuccessful laparoscopy due to extensive pelvic adhesions or spill of the follicular fluid during oocyte collection. A number of these patients were excluded from the study during determination of the accuracy of the ultrasonic monitoring of the follicle and during the assessment

Table 2.3.1 Reasons for exclusion for each item under investigation

Reasons for exclusion / Item	US unreliable	Lap. unreliable	At lap. one ovary inaccessible	No follicle by US and at lap.	No follicle by US; at lap. one or more follicles present	Ovulation occurred between US and lap.	Follicle seen by US; cyst seen at lap.	Patient not scanned by both observers	Both US measurements not performed by the same observer	Fewer follicles observerd by US than at lap.	Number of patients available for data analysis
Interobserver variation	4	8	–	6	5	1	1	15	–	–	10
Localization of the dominant follicle	4	8	1	6	–	1	–	–	–	–	30
Number of follicles in the left ovary	4	8	–	–	–	1	1	–	–	–	36
Number of follicles in the right ovary	4	8	1	–	–	1	1	–	–	–	35
Follicular size	4	8	–	6	5	1	1	–	–	–	25
Follicular growth rate	4	8	–	6	5	1	1	–	5	1	19

of follicular growth rate. The reasons for these exclusions and the number of patients ultimately available for data analysis are presented in Table 2.3.1.

Each patient underwent laparoscopy as part of the routine infertility work up. CC 100 mg was administered from day 5 to day 9 of the menstrual cycle and 5,000 IU HCG were given on day 13 to establish multifollicular growth and to time the moment of ovulation. Laparoscopy was performed 35 h following HCG administration, irrespective of the outcome of the ultrasound examinations.

A Diasonograph /EMI 4200 static B-compound scanner (Nuclear Enterprises Ltd, Edinburgh, Scotland) with internal focused 2.25 and 3.5 MHz probes was used. Ultrasound velocity was calibrated at 1,540 m /sec. Electronic calipers were employed to measure three follicular diameters at right angles to each other and the MFD was calculated (Hackelöer and Robinson, 1978; Hackelöer et al., 1979).

The reproducibility of the MFD measurements is expressed by the intra- and inter-observer variation. The intra observer variation was determined by 30 blind measurements of the MFD in three randomly chosen patients in which ultrasonic measurements of MFD were carried out within 2 h of laparoscopy by two independent observers. The accuracy of the ultrasonic technique was assessed by comparing ultrasonic and laparoscopic findings on the location of the dominant follicle, the number of follicles for each ovary and follicular size (MFD). Follicular fluid was aspirated under laparoscopic guidance. From the amount of fluid obtained, the MFD was estimated according to the formula:

$$\mathrm{MFD} = 2 \cdot \sqrt[3]{\frac{V}{4/3\pi}}$$

Follicles with a volume of less than 1 ml equivalent to an MFD of less than 12.4 mm were disregarded. A comparison of follicular size was only made for the largest follicle per ovary.

Follicular growth rate was calculated in mm /24 h. For practical reasons only the leading follicle was measured twice within a time interval of 10-17 h, the second measurement always being performed within 2 h prior to laparoscopy.

Results

1. Reproducibility

The intra- and inter-observer variation expressed as the pooled standard deviation, was 0.95 and 1.62 mm respectively.

2. *Accuracy*

Localization of the dominant follicle (n = 30, Table 2.3.1).
In 30 cases localization of the dominant follicle by ultrasound was possible. Localization was correct in 22 cases (73 %). Reasons for incorrect localization in the remaining 8 cases were: the dominant follicle was wrongly considered absent (5 cases); both ovaries contained a preovulatory follicle of about the same size (2 cases); a parovarian cyst was taken for a follicle (1 case).

Number of follicles for each ovary (n = 71, Table 2.3.1).
Since the number of follicles for each ovary was virtually the same (Table 2.3.1), the results were considered together (71 ovaries). For 51 ovaries (72 %) there was agreement between ultrasonic and laparoscopic findings. For 15 ovaries (21 %) a discrepancy of one follicle was found, for 5 ovaries (7 %) the discrepancy exceeded one follicle. These discrepancies were due to misinterpretation and overlook of small follicles.

Follicular size (n = 25, Table 2.3.1).
A total of 27 follicles in 25 patients was compared. The difference between the ultrasonically and laparoscopically determined MFD was more than 6 mm in three follicles (11 %), within 4 mm in 24 follicles (89 %) and within 2 mm in 13 follicles (48 %). Figure 2.3.1 shows the relationship between ultrasonic and laparoscopic measurements of follicular size. In 14 cases an overestimation, in 13 cases an underestimation by ultrasound was found.

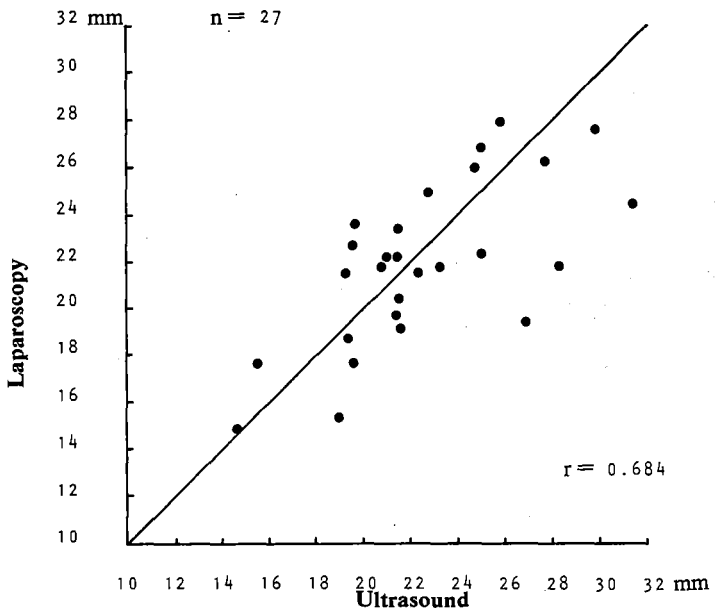

Fig. 2.3.1.
Follicular diameter measured by ultrasound and calculated from aspirated follicular volume

3. *Follicular growth*

Follicular growth rate during the last 10-17 h prior to laparoscopy (n = 19, Table 2.3.1). Follicular growth rate expressed in mm /24 h ranged from -3.2 to + 7.1 mm. The median was +3.9 mm (Fig. 2.3.2).

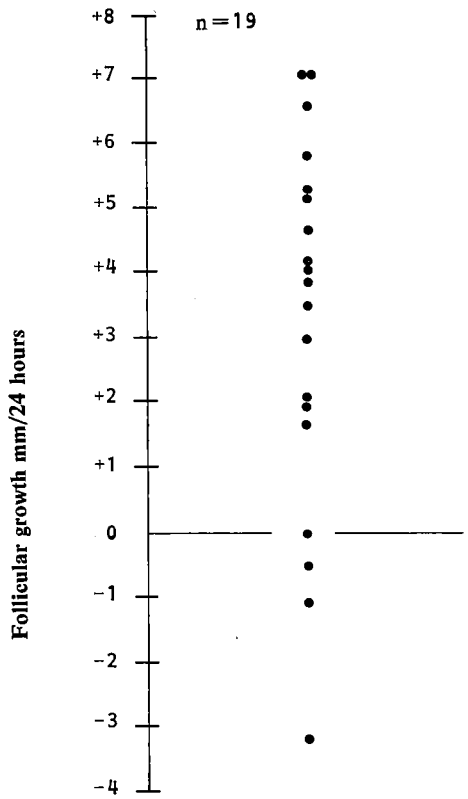

Fig. 2.3.2. Follicular growth in the last 10 - 17 h prior to laparoscopic oocyte collection

Discussion

The intra-observer variation showed a small pooled SD, indicating a high degree of intra-observer reproducibility. The considerably larger pooled SD for the interobserver variation indicates that evaluation of follicular growth should preferably be done by one investigator.

Localization of the dominant follicle by ultrasound was accurate in the majority of cases. Failures were usually a result of confusion between the true follicle and other cyctic structures e.g. parovarian cysts, fluid containing spaces between pelvic adhesions and hydrosalpinx. In these cases only serial measurements may provide a positive diagnosis of a growing Graafian follicle. Sometimes both ovaries show a preovulatory follicle rendering determination of the dominant follicle as very difficult. Accurate information can also be obtained on the number of follicles for each ovary. Only small follicles were missed which in some instances may have been caused by unfavourable position of the ovary due to pelvic adhesions or an overdistended bladder. In one patient two or more follicles were interpreted as one, most likely due to inappropriate setting of the ultrasound equipment.

A close agreement between follicular size as determined by ultrasound and by laparoscopy in the present study was found, although even better results have been reported elsewhere (O'Herlihy et al., 1980b; Kerin et al., 1981). This slight discrepancy may well be explained by the high percentage of pelvic adhesions (56 %) in our material and may also account for the rather wide range in follicular growth rate values during the last 10-17 h prior to laparoscopy. The latter data therefore do not permit any conclusions as to the rate of follicular growth during this period of time. Obviously, a larger number of patients is needed to answer this particular question.

2.4 Comparison of Graafian follicle dimensions as determined by static and real-time sector scanning

R.A. Leerentveld, I. van Gent, M. van der Stoep, A.Th. Alberda, J.W. Wladimiroff

Department of Obstetrics & Gynaecology, Erasmus University Rotterdam, The Netherlands

Published in Fertility and Sterility 42:929, 1984
(Reproduced with permission of the publisher, the American Fertility Society)

Introduction

In a previous study (Leerentveld et al., 1983), data were provided on reproducibility and accuracy of measurements of preovulatory Graafian follicles using static ultrasound. In this study, a close agreement was observed between MFD dertermined by ultrasound and derived from follicular volumes obtained during laparoscopic oocyte collection. Similar findings were described by O'Herlihy et al. (1980b) and Kerin et al. (1981). Whereas the latter only employed a static scanner, O'Herlihy and associates (1980b) used both static and real-time linear-array scanning, without, however, considering the data from each scanning

technique separately. To date no comparative studies between static and real-time sector scanning in the estimation of follicular size have been reported. Only in-vitro experiments by Wetzels (1983) demonstrated that the accuracy of static scanning in measurements of volumes of small objects was significantly higher than that of the real-time sector scanning. The objective of the present study was to compare the accuracy of follicle measurements by static and real-time sector scanning techniques, both in relation to follicular size during laparoscopy.

Materials and methods

Sixty-nine patients awaiting laparoscopic oocyte aspiration for IVF were studied in 91 cycles. All had CC stimulated cycles (50-100 mg /day for 5 days, starting on day 4, 5, or 6 of the menstrual cycle), and ovulation was timed by 5,000 IU HCG given 35 h prior to laparoscopy.

In 34 patients (56 cycles; group I), laparoscopy was performed for the sole purpose of establishing IVF. Follicular maturation was monitored by serial plasma E2 estimations and repeated ultrasonic follicle measurements. HCG was administered at the time ultrasound revealed a predominant follicle with an MFD of 20 mm or more, and urine LH was < 100 IU /l. In 26 of 56 cycles, laparoscopy had to be cancelled for reasons presented in Table 2.4.1 Thirty-five patients (35 cycles; group II) underwent laparoscopy irrespective of the ultrasound findings during a routine infertility work up. No serial plasma E2 estimations or urine LH determinations were carried out. In this group of patients, laparoscopic evaluation of tubal patency was combined with oocyte collection for IVF. Fourteen cycles were excluded for reasons presented in Table 2.4.1. Altogether, 51 cycles were left for data analysis (group I, 30 cycles; group II, 21 cycles). In each patient ultrasonic follicle measurements were carried out within 2 h before laparoscopy using a Diasonograph NE 4200 static scanner (Nuclear Enterprises Ltd., Edinburgh, Scotland) with an internally focused 3.5 MHz probe and a Kretz Combison 100 real-time sector scanner (Kretztechnik GmbH, Zipf, Austria) using a 2.5 MHz transducer. Because the patients had to fast prior to laparoscopy, the required full bladder was achieved by instillation of 0.9 % saline solution through an indwelling catheter. Ultrasound velocity was calibrated at 1,540 m / sec. Electronic calipers were employed to measure three diameters at right angles to each other and the MFD was calculated. Complete aspiration of follicular contents was attempted under laparoscopic guidance. From the volume obtained, the MFD was calculated according to the formula:

$$MFD = 2 \cdot \sqrt[3]{\frac{V}{4/3\pi}}$$

Table 2.4.1. Reasons for Exclusion in Both Groups Under Investigation

		Group I (56 cycles/34 patients)		Group II (35 cycles/35 patients)	
a	Inadequate folliculogenesis judged by ultrasound	11*	Laparoscopy canceled	3	Laparoscopy performed
b	Spontaneous ovulation as determined by ultrasound	4*		2	
c	Insufficient plasma E_2 levels	4*		—	
d	Onset of the LH surge prior to planned HCG administration	5*		—	
e	Laparoscopy unreliable or spill of follicular contents	4	Laparoscopy performed	7	
f	Incomplete data on follicular volume	1		1	
g	Ultrasound judged unreliable	1		1	

*In four cycles laparoscopy was canceled for more than one reason mentioned.

A comparison of follicular size by ultrasound and laparoscopy was only carried out for the largest follicle per ovary. From the 51 cycles available, 34 cycles with a total of 42 follicles were scanned by one observer, and 17 cycles with a total of 18 follicles were studied by two observers. In the latter instance, both measurements were taken into account, resulting in 36 observations. Correlation coefficients were calculated on paired data from static scanning versus laparoscopy, real-time sector scanning versus laparoscopy, and static scanning versus real-time sector scanning.

Results

Figure 2.4.1 presents the data of follicular size for static scanning versus laparoscopy. The data on follicular size for real-time scanning versus laparoscopy are given in Figure 2.4.2. Figures 2.4.1 and 2.4.2 demonstrate that there is a

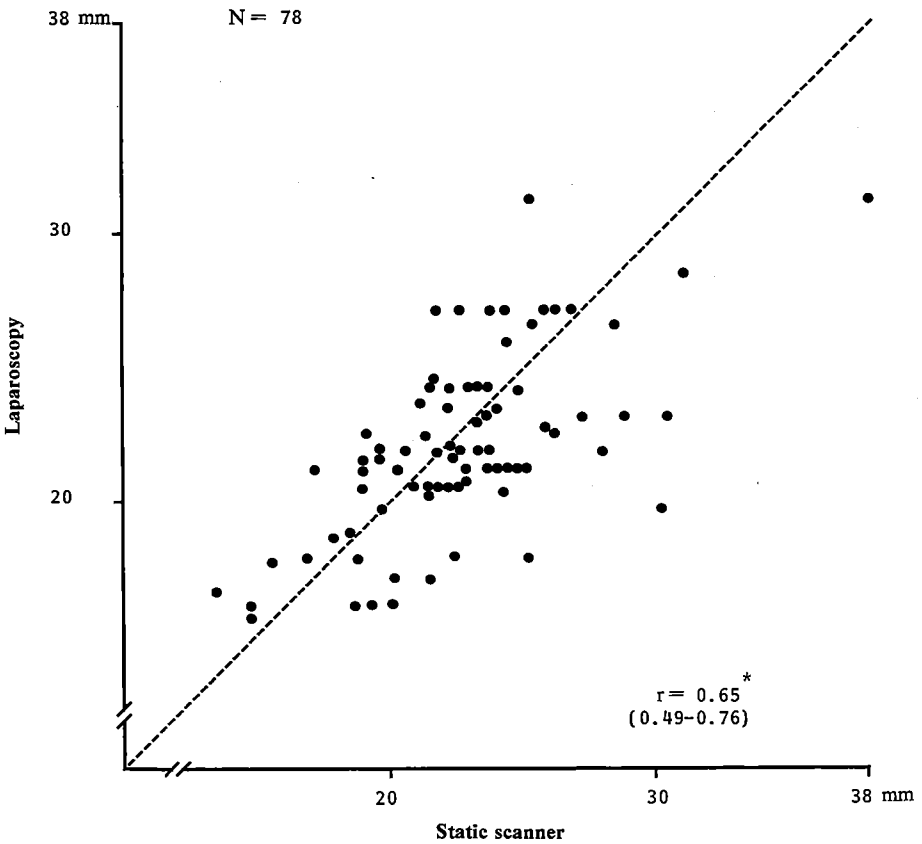

Fig. 2.4.1
Static scanning versus laparoscopy in the measurement of follicular size *P < 0.001

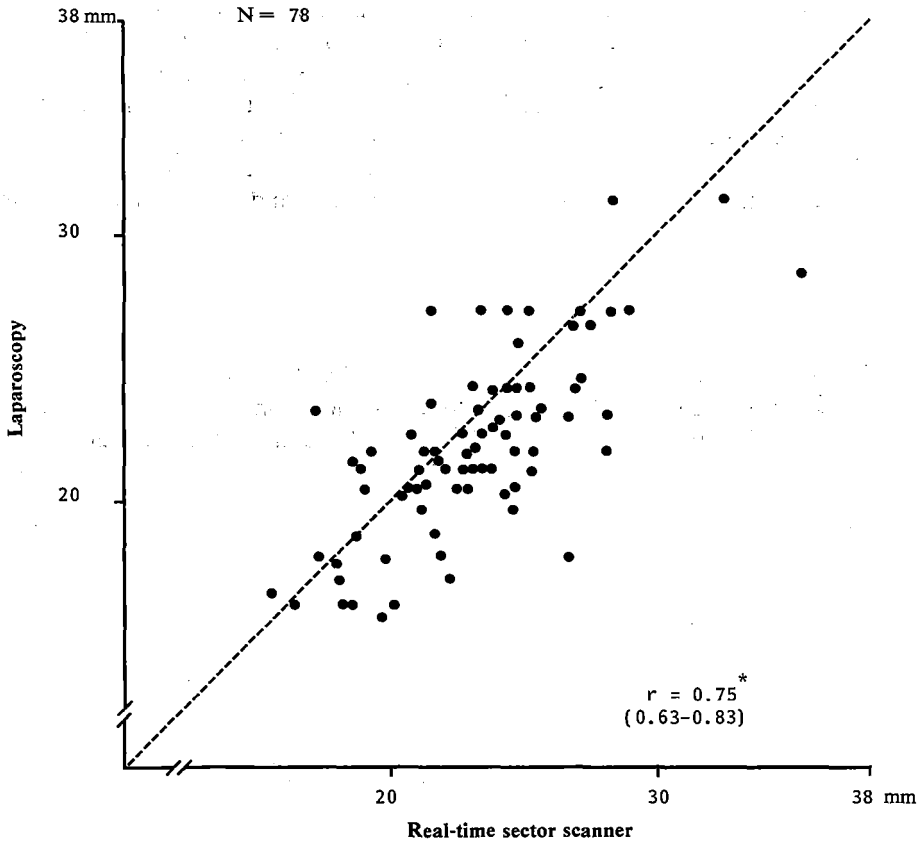

Fig. 2.4.2
Real-time sector scanning versus laparoscopy in the measurement of follicular size. *P < 0.001

considerable overlap in 95% confidence intervals for both scanning techniques versus laparoscopy. A close correlation was established for both ultrasound techniques under investigation ($r = 0.86$; $P < 0.001$).

Discussion

In early studies, ultrasonic monitoring of follicular growth has always been carried out by static scanning techniques (Hackelöer et al., 1979; Kerin et al., 1981). In later studies linear-array real-time techniques were also introduced in measurements of follicular size (O'Herlihy et al., 1980a) mainly for time-saving reasons. Many authors agree, however, that the resolution of linear-array scanners is inferior to that of static scanning equipment. No in-vivo data are available on the accuracy provided by real-time scanners. However, a recent

in-vitro experiment by Wetzels indicated a certain degree of superiority of static over real-time scanning in the determination of follicular size.

In the present in vivo study a slightly better correlation was established between findings at laparoscopy and measurements of follicular size when employing real-time sector equipment. The latter equipment did, however, show a slight systematic overestimation of follicular size (Fig. 2.4.2) as opposed to the static scanner (Fig. 2.4.1), as was also demonstrated in a previous study (Leerentveld et al., 1983). The slightly better results in the present study using real-time sector equipment are probably determined by the more accurate planar adjustment of the real-time transducer, proper selection of the optimal scanning plane, and exclusion of the influence of breathing and bowel movements on the ultrasonic image.

In our unit, static scanning of Graafian follicles is at present exclusively employed for the interpretation of structures within the follicle.

2.5 Conclusions

Both from literature and the studies performed in our centre, it may be concluded that static B-compound as well as real-time sector scanning techniques provide accurate and reproducible information on the presence of follicles, their localization, size and growth.

Data on the accuracy and reproducibility of transvaginal follicle measurements are scarce. Yee and associates (1987) describe improvement of ultrasound images by transvaginal scanning in 87 % of patients with suboptimal transabdominal ultrasonography. A significant correlation was observed between follicular fluid volume and the MFD determined transvaginally.

Since ultrasound probes designed for vaginal application generally transmit a higher frequency (5-7 MHz) than transabdominal transducers, a higher resolution in the near field may be expected enabling more accurate measurements in this area. Moreover, as the vaginal transducer approaches normally situated ovaries much closer than at transabdominal scanning, this will result in an increased accuracy of follicle measurements.

CHAPTER 3

FOLLICULAR GROWTH IN STIMULATED CYCLES FOR IN-VITRO FERTILIZATION

3.1 Literature on follicular growth in stimulated cycles for in-vitro fertilization

3.1.1 *Follicular growth in clomiphene stimulated cycles for in-vitro fertilization*

In Section 1.1.2 studies on the ultrasonographical aspects of follicular growth in the induced menstrual cycle were discussed. In the majority of these studies ovulation induction was carried out in anovulatory patients.

Papers on the characteristics of follicular growth in CC stimulated cycles for IVF in regularly cycling women are scarce. In 1981 Hoult et al. reported on three pregnancies achieved in 120 CC stimulated cycles in regularly cycling women which were only monitored by ultrasound. HCG was administered at a MFD of 18 mm calculated from three maximal diameters at right angles to each other. However, no data on follicular growth rate or ultimate preovulatory follicular size were provided. Trounson et al. (1981) compared results in spontaneous cycles with those obtained in CC stimulated cycles. On a total of 34 ET's, four normal pregnancies were achieved, all resulting from a stimulated cycle. Also here, no data on follicular growth were presented. Vargyas et al. (1982) found a linear increase in diameter of the dominant follicle in CC stimulated cycles up to the moment of HCG administration. The mean daily increase in follicular diameter in the four days preceding HCG administration amounted to 2.5 mm/24 h and the mean follicular diameter at HCG administration was 22.1 mm. Buttery et al. (1983) reported on 203 CC stimulated cycles, resulting in nine pregnancies. Using real-time linear array equipment and measurements of the mean of three maximal follicular diameters, the average growth rate of the largest follicle was 1.6 mm/24 h. The MFD of the largest follicle at the time of the LH surge was 19 mm, with a range from 15 to 23 mm. These authors advocated E2 determinations as a primary monitoring method and used ultrasound and cervical mucus changes only to assist the interpretation of oestrogen patterns. Monitoring of follicular development by ultrasound alone was judged inadequate for an IVF program. Finally, Hünlich et al. (1984) considered the ultrasonic characteristics in 23 CC stimulated cycles resulting in clinical pregnancies. Both the daily follicular diameter measured in two directions, and plasma E2 concentration increased in a linear fashion; no correlation between paired observations was found. Nevertheless, it was concluded that detection of the linear growth of the leading follicle and corresponding E2 level were the keys to well-timed HCG induction of ovulation.

From the available data it may be concluded that the dominant follicle in CC stimulated IVF cycles grows in a linear fashion at a rate of 1.6 to 2.5 mm /24 h. The ultimate preovulatory diameter ranges from 19.0-22.1 mm, although successful ovulation has been documented from an MFD of 15 mm onwards.

3.1.2 *Follicular growth in clomiphene citrate/human menopausal gonadotrophin stimulated cycles and in cycles stimulated using human menopausal gonadotrophin, follicle stimulating hormone or a combination of both compounds for in-vitro fertilization*

As soon as it was recognized that IVF treatment results were positively correlated with the number and quality of replaced embryos (Edwards et al., 1984; Jones et al., 1984; Testart et al., 1985), superovulation protocols were designed using a combination of CC and HMG or HMG alone. Studies of these cycles have mainly focused on mean oocyte harvest and conception rates, while much less attention was paid to the ultrasonographical characteristics of follicular growth.

Bayly et al. (1985) compared three HMG stimulation protocols, two of which combined with CC. Apart from the higher incidence of an endogenous LH surge in the low dose CC group, there were no significant differences in the length of the follicular phase, the number of preovulatory follicles, collected and fertilized oocytes, and ET and pregnancy rates. Testart et al. (1985) also employing a combination of CC and HMG, administered HCG on basis of the E2 value per follicle exceeding 16 mm in diameter. In both these studies no data were given on follicular growth. Vargyas et al. (1984) compared three different stimulation schemes: CC only, HMG only and a combination of CC and HMG. It was concluded that optimal follicular development and fertilization rates were obtained using the combination of CC /HMG rather than a single agent. Diamond and coworkers (1986) also compared these three stimulation regimens and found CC /HMG to be superior in terms of follicular development, oocyte recovery and embryo transfer. Hill et al. (1987), however, could not establish any significant difference in the outcome of five different CC /HMG treatment protocols. In all instances the administration of HCG was scheduled on basis of ultrasonographical findings and plasma E2 levels. Detailed information on ultrasonographical aspects of follicular growth, however, was not given.

In 1983 two groups reported on successful ovarian stimulation using HMG stimulation only. Jones and coworkers (1983) from Norfolk planned the administration of HCG on the type of E2 response (high, intermediate or low) and the "biologic shift" (as determined by vaginal cytology, the quality and amount of the cervical mucus, and dilatation of the external cervical os). Ultrasound served only a complementary role, except in patients who had a positive history of conization of the cervix. The ultrasound data of the Norfolk group were summarized by Mantzavinos et al. (1983). Using their protocol for

stimulation and monitoring, the range of follicular diameters was 16.1-18.5 mm on the day before follicle aspiration, depending on the localization of the dominant follicle in the right or left ovary. No mention was made of the methodology of follicle measurements. In the intermediate responding patient group a daily increase in follicular diameter of 1.6 mm was found. Laufer et al. (1983b) also employed HMG stimulation. In their programme ultrasonography, and not the E2 level, was considered a more meaningful parameter of follicular development. In their opinion, the value of E2 in the prediction of the number of developing follicles in stimulated cycles is rather limited (Laufer et al., 1983a). HCG was administered when at least two large follicles (16-18 mm) were visualized. Data on follicular growth patterns in their HMG stimulated cycles were, however, not presented. Pregnancy rates were similar to those observed in the Norfolk programme (16 vs. 19%). Recently, Hull and associates (1986) reported on HMG stimulated cycles in 38 patients monitored by serum E2 levels and cervical mucus scoring. Ultrasound examination was only performed at the time of HCG administration, no data on follicular growth were therefore available. Their results indicate a good correlation between total follicular volume of both ovaries or the total follicular volume of the ovary containing the dominant follicle and preovulatory E2 levels on the day of HCG administration. However, the size or volume of the largest follicle was not related to E2 levels.

Recently, a combination of FSH and HMG for ovarian stimulation was studied (Bernardus et al., 1985; Muasher et al., 1985). The data indicate that this combined treatment protocol results in a high pregnancy rate (23.0 % per follicle aspiration). Favourable results were particularly obtained in a group of 23 patients which had responded poorly to HMG /HCG during previous attempts. In neither study data on follicular growth were given. Russell et al. (1986) compared ovarian hyperstimulation by FSH and HMG. Their results are in favour of FSH stimulation, but the number of patients was low and differences did not reach statistical significance. Data on ultrasonographical findings were not presented.

In summary, although a vast literature exists on the use of gonadotrophins, both alone and in combination with CC, little is known about follicular growth and preovulatory follicular dimensions as determined by ultrasound when using these compounds. It is only a clinical impression that follicular growth rate values do not differ significantly from those observed in cycles stimulated with CC only. It seems that the ovulating dose of HCG can be administered in the presence of smaller follicles (MFD 16-19 mm) than during stimulation with CC.

3.2 Introduction to own observations

Within the Academic Hospital Rotterdam-Dijkzigt IVF programme, two studies were undertaken during CC stimulated cycles in normally cycling women, to assess the growth of the dominant follicle, both under monofollicular and multifollicular conditions preceding and following HCG administration. Where possible, growth patterns were compared with those observed in spontaneous cycles.

3.3 Ultrasonographic assessment of Graafian follicle growth under monofollicular and multifollicular conditions in clomiphene citrate stimulated cycles

R.A. Leerentveld, I. van Gent, M. van der Stoep, J.W. Wladimiroff

Department of Obstetrics & Gynaecology, Erasmus University Rotterdam, The Netherlands

Published in Fertility and Sterility 43:565, 1985
(Reproduced with permission of the publisher, the American Fertility Society)

Summary

Growth of the dominant follicle was assessed by means of real-time sector scanning in 52 CC stimulated cycles in 44 patients awaiting laparoscopic oocyte recovery for IVF purposes. Follicular growth profiles under both monofollicular and multifollicular conditions were compared with each other and with a reference group curve derived from 26 ovulatory cycles in 25 spontaneous cycling women. In the CC stimulated cycles, growth of the dominant follicle under both monofollicular and multifollicular conditions was significantly faster ($P < 0.01$) than in the reference group. There was no significant difference in follicular growth between monofollicular and multifollicular CC stimulated cycles.

Introduction

In the recent decade, ultrasonographic observation of Graafian follicle growth and ovulation established more precise insight in the physiology of follicular growth and ultimate follicular dimensions at ovulation, both in spontaneous and induced menstrual cycles (Hackelöer et al., 1979; Ylöstalo et al., 1979; Hackelöer et al., 1980; Nitschke-Dabelstein et al., 1980; Renaud et al., 1980; O'Herlihy et al., 1980a; Queenan et al., 1980; Kerin et al., 1981; Seibel et al., 1981; O'Herlihy et al., 1982a, 1982b; Sallam et al., 1982; Vargyas et al., 1982; Smith et al., 1983; Wetzels, 1983). In an early publication Hackelöer et al. (1979) observed a decrease in follicular size following the LH surge. In a later publication (Hackelöer et al., 1980), however, they found like most other workers (O'Herlihy et al., 1980a; Renaud et al., 1980; Kerin et al., 1981), a linear increase in follicular diameter during the last five days prior to ovulation. The follicular growth rate ranged from 1.5 to 3.1 mm/24 h in this particular period, and the ultimate preovulatory diameter was 18 to 24 mm. Only Queenan et al. (1980) established an exponential follicular growth profile up to the supposed moment of ovulation.

The conduct of the dominant follicle in CC stimulated cycles, both under monofollicular and multifollicular conditions, remains controversial. Vargyas and

associates (1982) found a mean daily growth rate (2.4 mm/24 h) and a preovulatory mean diameter of the dominant follicle (22.1 ± 0.4 mm SEM) within limits for spontaneous ovulatory cycles as presented above. However, no comparison with a normally cycling reference group was made. Whereas Smith et al. (1983) found no apparent differences in follicular size in a CC stimulated group, compared with a normal cycling control group, O'Herlihy and coworkers (1982a) demonstrated a slight but significant increase in follicular dimensions at ovulation in CC stimulated cycles, although selection of the dominant follicle seems to occur later in the follicular phase. Wetzels (1983), however, only found an accelerated growth of the dominant follicle in CC induced cycles in the presence of more than one follicle. After subtraction of the multifollicular cycles, the follicular growth profile did not differ significantly from the growth rate in spontaneous cycles.

Data on growth and preovulatory dimensions of the dominant follicle in gonadotrophin stimulated cycles are scarce and confusing (Ylöstalo et al., 1979; Nitschke-Dabelstein et al., 1980, Seibel et al., 1981; O'Herlihy et al., 1982b; Sallam et al., 1982)

The objective of the present study was to elucidate follicular growth profiles in CC stimulated cycles under both monofollicular and multifollicular conditions, relative to spontaneous growth profiles.

Materials and methods

A Kretz Combison (Kretztechnik GmbH, Zipf, Austria) real-time sector scanner with a 2.5 MHz transducer was employed. Integral electronic calipers were used, and ultrasound velocity was calibrated at 1,540 m/sec. Maximum follicular dimensions were determined in three planes perpendicular to each other, and a MFD was calculated, as described by Hackelöer et al. (1979).

Normal follicular growth profiles were determined in 26 ovulatory cycles in 25 regularly cycling women (28 ± 2 days). All subjects in this reference group were scanned at two daily intervals from day 9 or 10 of the menstrual cycle. When a follicle reached an MFD of ≃ 15 mm, daily scans were performed until ultrasonographic evidence of ovulation was established (Wetzels, 1983). All data were plotted against the day preceding the day on which ovulation first became apparent (day 0).

Forty-nine normally cycling patients with irreparable tubal damage or absent Fallopian tubes awaiting laparoscopic oocyte recovery for IVF purposes were studied in 57 cycles. All cycles were CC stimulated (50-100 mg/day for 5 days, starting on day 4, 5, or 6 of the menstrual cycle) for induction of multifollicular growth. 5,000 IU of HCG were administered when ultrasonographic examination revealed a dominant follicle with an MFD of 20 mm or more. Laparoscopic oocyte recovery was scheduled 35 to 36 h after HCG administration. In nine cycles monofollicular development occurred as established during laparoscopy (group I). Follicle aspirates < 1.0 ml, corresponding with an MFD

of 12.4 mm, were not taken into account (Leerentveld et al., 1983). In 20 cycles bifollicular (group II) and in 18 cycles trifollicular (group III) growth occurred. In ten cycles four or more follicles developed; these were considered together (group IV).

Only growth of the dominant follicle was determined, because distortion of smaller follicles due to irregular flattening precludes accurate measurements. Five cycles had to be excluded from the study because of insufficient data on ultrasonographically determined follicular size, leaving 52 cycles in 44 patients for data analysis (group I, 8 cycles; group II, 19 cycles; group III, 15 cycles; group IV, 10 cycles).

All subjects were scanned at two daily intervals from day 9 or 10 of the menstrual cycle. As soon as the dominant follicle reached an MFD of 15 mm, daily observations were made until HCG administration. All ultrasonographic follicle measurements were plotted against the day of laparoscopic oocyte recovery (day 0). Both in the reference group and in groups I to IV, data on follicular dimensions were considered on day 0, day -1, day -2, and day -3, because the number of measurements before this period was too small in the CC stimulated group to allow further analysis. The MFD $\pm$ 1 SD was calculated in the reference group and in groups II and III for each observation day. Because the number of observations in groups I and IV was too small to allow statistical analysis, single measurements are presented.

The Mann-Whitney two-sample rank sum test was used for statistical evaluation of the data on follicular size for the reference group and all four groups under investigation for each observation day separately.

Table 3.3.1
Mean Follicular Diameter (mm) in the Reference Group and the combined Groups I to IV during days -3 to 0*

	Day -3	Day -2	Day -1	Day 0
Reference group	16.8 $\pm$ 2.8	18.7 $\pm$ 2.6	19.7 $\pm$ 2.1	21.6 $\pm$ 2.0
CC stimulated; groups I-IV	18.7 $\pm$ 2.5	21.3 $\pm$ 1.6	23.2 $\pm$ 1.9	25.2 $\pm$ 2.6
Statistical significance	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$

*Mean $\pm$ SD

Results

Table 3.3.1 compares data (mean $\pm$ SD) on follicular size from the reference group with combined data from groups I to IV. The average size of the dominant follicle in CC stimulated cycles is significantly larger ($P < 0.01$) than that in spontaneous cycles, except on day -3.

In Figures 3.3.1 to 3.3.3, data on follicular size derived from group I (single values; Fig. 3.3.1), follicular growth curves from groups II and II (mean ± 1 SD; Fig. 3.3.2), and data from group IV (single values; Fig. 3.3.3) are presented relative to the reference group (mean ± 1 SD). Dimensions of the dominant follicle in groups I to III are significantly larger than those observed in the reference group ($P < 0.01$) from day -2 to day 0 and those in group IV only for day -1 ($P < 0.05$) and day 0 ($P < 0.01$). On day -3 no significant differences were found between groups.

In Figure 3.3.4 the mean follicular growth curves ± 1 SD of the dominant follicle in the bifollicular and trifollicular situation are presented with single data on follicle size from group I. Statistical analysis of the data of group I against those of groups II and III showed no significant differences on any of the observation days.

In Figure 3.3.5 the mean follicular growth curves ± 1 SD of groups II and III are compared with the single values from group IV. The values of the dominant follicle when four or more follicles are present show a lower growth profile in comparison with the bifollicular and trifollicular situation. The MFD on days -2 and -1 from group IV is significantly smaller ($P < 0.05$) when compared with group III. None of the other differences between group IV and groups II and III reached statistical significance.

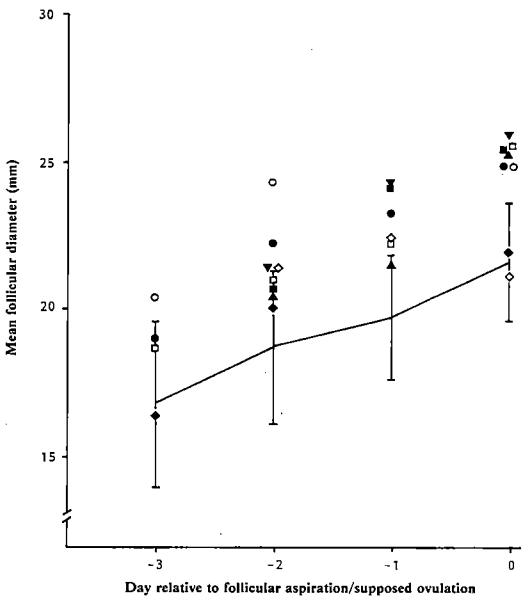

Fig. 3.3.1
Individual data of group I relative to the reference group (mean ± 1 SD)

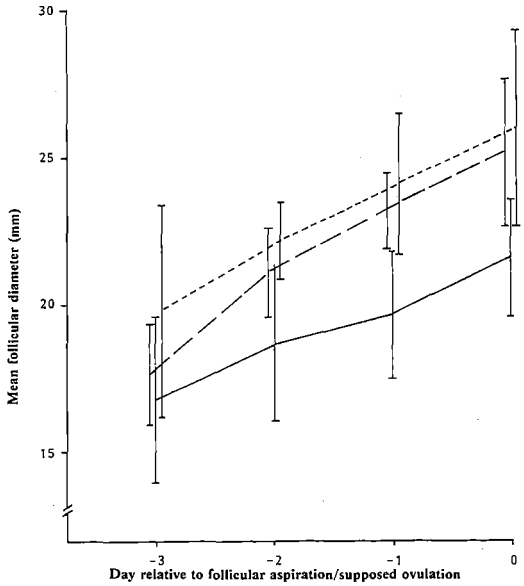

Fig. 3.3.2
Mean (± 1 SD) growth curves of group II (– – –) and group III(-------) relative to the reference group

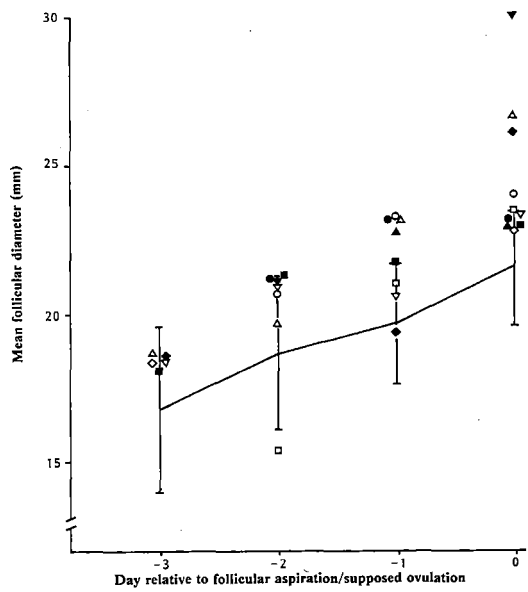

Fig. 3.3.3
Individual data of group IV relative to the reference group (mean ± 1 SD)

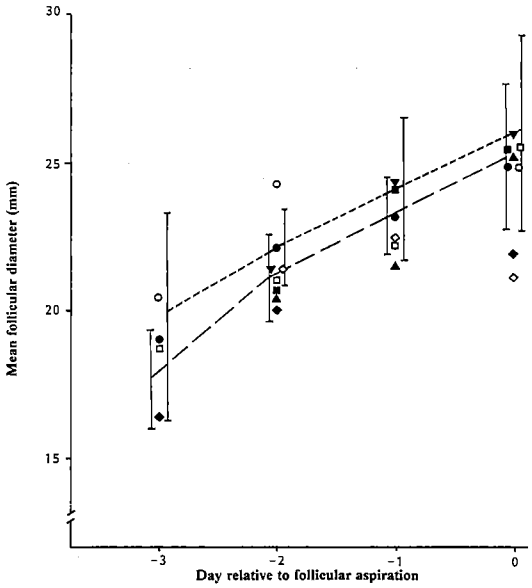

Fig. 3.3.4
Individual data of group I relative to group II (– – –) and group III (--------)
(mean ± 1 SD)

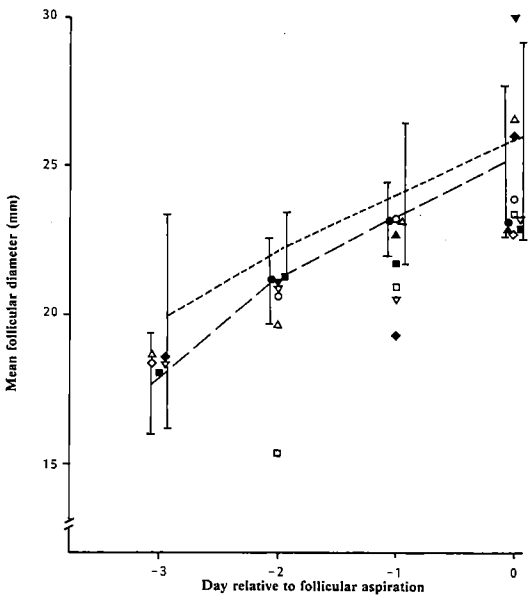

Fig. 3.3.5
Individual data of group IV relative to group II (– – –) and group III (--------)
(mean ± 1 SD)

Discussion

To date, in only two studies are data on follicular growth rate in spontaneous and CC induced cycles as determined by ultrasound compared. Both O'Herlihy (1982a) and Wetzels (1983) found a faster but still linear growth profile in CC induced cycles as compared with spontaneous cycles. Similar findings were obtained in the present study. Dimensions of the dominant follicle were significantly larger from days -2 to 0 in groups I to III and for days -1 and 0 in group IV as compared with the reference group. In contrast to O'Herlihy et al (1982a) and Wetzels (1983), HCG was administered in all CC cycles approximately 35 h prior to laparoscopy. It is not known, however, whether HCG administration may have any effect of follicular growth in this particular period of the menstrual cycle.

Whereas Wetzels (1983) observed sifnificantly larger follicles in the CC stimulated group as early as day -5, O'Herlihy et al. (1982a) could only establish this difference for days -1 and 0. Our data are more in agreement with those obtained in the latter study, because we found significant differences from day -2 in groups I and III and from day -1 in group IV. Discrepancy also exists in follicular growth profiles in monofollicular CC stimulated cycles. Whereas in Wetzels study (1983) the mean follicular growth curve derived from monofollicular cycles was not significantly different from that in spontaneous cycles, we observed significantly larger follicles also in the monofollicular CC stimulated cycles for days -2, -1 and 0. Our finding may be explained by increased release of gonadotrophins as a result of CC administration (Jacobson et al., 1968; Wu, 1977). This is supported by the finding of raised plasma E2 levels in CC stimulated cycles, compared with spontaneous cycles (Smith et al., 1983).

Because under monofollicular and multifollicular conditions a wide range in individual growth profiles and ultimate preovulatory follicular dimensions was found, the use of ultrasound as a single method in the assessment of follicular development in the planning of oocyte retrieval may be inappropriate, as has been suggested by others (Garcia et al., 1983a, 1983b; Buttery et al., 1983). Moreover, in stimulated cycles there is a limited association between follicular size and oocyte maturity (Carson et al., 1982). Combined evaluation of ultrasonographic data with plasma E2 values has been reported to improve planning of follicle aspiration and harvesting of mature oocytes for IVF (Garcia et al., 1982a, 1982b).

3.4 24-h echographic profile study on follicular growth prior to laparoscopic aspiration of the Graafian follicle in clomiphene citrate/human chorionic gonadotrophin stimulated cycles

R.A. Leerentveld, J.W. Wladimiroff

Department of Obstetrics & Gynaecology, Erasmus University Rotterdam, The Netherlands

Published in the European Journal of Obstetrics, Gynecology and Reproductive Biology 22:287, 1986

Summary

Follicular growth in the last 24 h preceding laparoscopic oocyte pickup for IVF purposes was studied in 46 CC/HCG stimulated cycles by means of real-time sector ultrasound. A linear increase in MFD of 2.5 mm was established. Growth rates under monofollicular and multifollicular conditions did not differ significantly. However, the absolute dimensions of the dominant follicle in the multifollicular subgroup exceeded those observed in the monofollicular subgroup ($P < 0.01$) for each observation point. A fixed CC/HCG treatment scheme results in a wide range of ultimate preovulatory follicular dimensions, without any correlation between the individual follicular size and growth rate values ($r = 0.075$).

Introduction

At present a number of studies on follicular growth rate have been published (Hackelöer and Robinson, 1978; Hackelöer et al., 1979; Renaud et al., 1980; O'Herlihy et al., 1980a, 1982b; Queenan et al., 1980; Kerin et al., 1981; Bryce et al., 1982; Vargyas et al., 1982; Mantzavinos et al., 1983). In all instances growth curves were assessed by means of B-compound or real-time ultrasonography. Data derived from spontaneous, as well as stimulated menstrual cycles indicated a daily linear increase in follicular diameter, at least until the day of the LH surge. This increase ranged from 1.5 to 2.7 mm/24 h. Data on the behaviour of the dominant follicle during and after the LH surge do not show consistency. Whereas some report a constant size or even a decrease in follicular diameter (Hackelöer et al., 1979; Honda et al., 1984), in other studies a linear increase (Hackelöer and Robinson, 1978; O'Herlihy et al., 1980a, 1982b; Kerin et al., 1981; Bryce et al., 1982; Vargyas et al., 1982; Mantzavinos et al., 1983) or even an exponential increase in follicular diameter was established (Queenan et al., 1980).

No data are available on follicular growth after HCG administration in CC stimulated cycles. Knowledge about follicular growth in these circumstances may be of practical interest in the planning of oocyte recovery for IVF.

The objective of the present study is to elucidate the growth pattern of the dominant follicle following HCG administration in CC stimulated cycles.

Materials and methods

In the present study 71 patients underwent screening laparoscopy during their routine infertility work-up, combined with attempted oocyte recovery. Twenty-five patients were excluded from the study for various reasons, as presented in Table 3.4.1, leaving 46 patients for data analysis. All these individuals had regular, ovulatory cycles (26-30 days), established by biphasic BBTs and post-ovulatory midluteal serum P values (> 30 nmol/l). All cycles were stimulated by 50 mg CC a day for 5 days, starting on day 4, 5 or 6 of the menstrual cycle, depending on the day laparoscopy was scheduled. 5,000 IU HCG were administered 4 days following the last CC intake. This was followed by laparoscopy 34 h later, irrespective of the ultrasound findings.

Table 3.4.1
Reasons for exclusion

	Number of patients
Ultrasound judged unreliable	5
Insufficient bladder filling	2
Absence of folliculogenesis	5*
Ovulation during the observation period as judged by ultrasound	13**

* Pre-existent oligo/amenorrhoea in four cases
** Confirmed by the presence of a corpus haemorrhagicum at laparoscopy in 11 cases

All ultrasound observations were carried out by R.A.L. at 24, 16, 8 and 1 h prior to laparoscopy, employing a Kretz Combison 100 (Kretztechnik GmbH, Zipf, Austria) real-time sector scanner with a 2.5 MHz transducer. Integral electronic calipers were used, and ultrasound velocity was calibrated at 1,540 m/sec. Maximum follicular dimensions were determined in three perpendicular planes and an MFD was calculated as described by Hackelöer et al. (1979). Follicles with an MFD below 12.4 mm, corresponding to a follicular volume

of less than 1 ml, were disregarded. Intra-observer variance as expressed by the pooled SD amounted to 0.65 mm. Under multifollicular conditions only the growth of the dominant follicle was considered. A follicular growth curve was calculated from individual data on follicle size for the entire study group and for the monofollicular and multifollicular subgroups separately.

The Wilcoxon signed rank test was used for statistical evaluation of the data on follicular growth per observation period; the Mann-Whitney U test was employed for analysis of the differences in follicular size between both subgroups. The correlation between the size of the individual follicles at 1 h prior to laparoscopy and their 24 h growth rates was calculated using the Spearman rank correlation test.

Results

Figure 3.4.1 presents the follicular growth curves both for the entire study group (n = 46) and the monofollicular (n = 18) and multifollicular (n = 28) subgroups. Absolute figures on follicular size for each observation point are

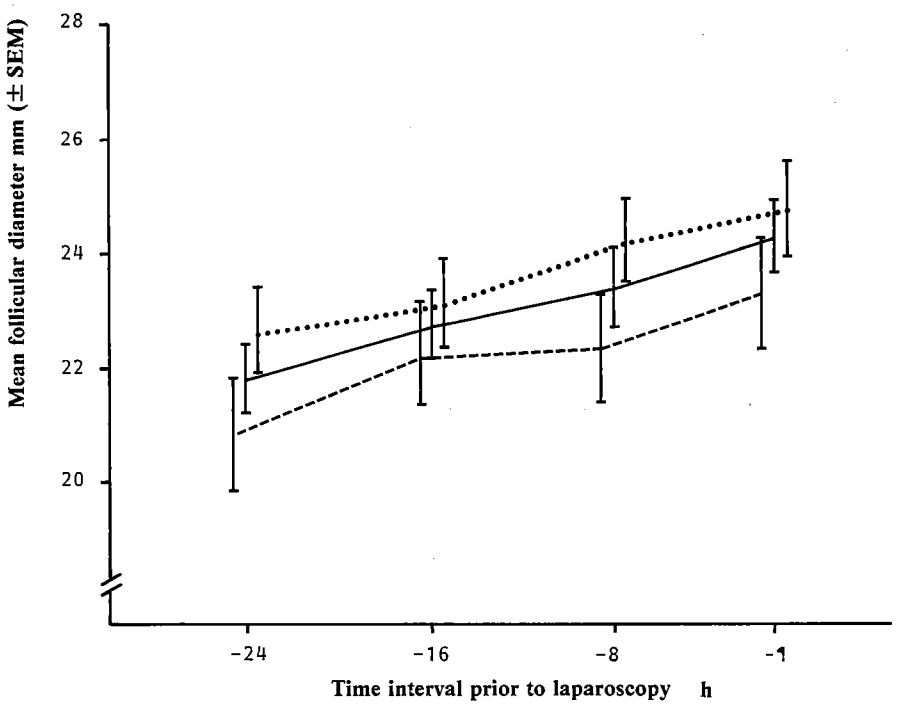

Fig. 3.4.1
Follicular growth curves (means ± SEM) for the entire study group (——), and the monofollicular (--------) and multi follicular (.....) subgroups

given in Table 3.4.2. Follicular growth for the entire study group shows an almost linear increase during the 23 h study period, resulting in a mean follicular growth rate of 2.5 mm /24 h. In the monofollicular and multifollicular subgroup a similar growth pattern was observed. The mean follicular growth rate in these

Table 3.4.2
MFD (mm) for each observation point
Data are means ± SEM $^{a}P < 0.01$ $^{b}P < 0.01$ $^{c}P < 0.01$ $^{d}P < 0.01$

	− 24 h	− 16 h	− 8 h	− 1 h
Entire study group (n = 46)	21.8 ± 0.65	22.7 ± 0.59	23.4 ± 0.64	24.2 ± 0.64
Monofollicular subgroup (n = 18)	20.8 ± 0.99[a]	22.2 ± 0.90[b]	22.3 ± 0.94[c]	23.3 ± 0.95[d]
Multifollicular subgroup (n = 28)	22.6 ± 0.83[a]	23.1 ± 0.78[b]	24.2 ± 0.84[c]	24.8 ± 0.84[d]

two subgroups was 2.6 and 2.3 mm /24 h respectively; this difference is not statistically significant. The difference in the mean values for the MFD between the monofollicular and multifollicular subgroup was statistically significant ($P < 0.01$) for each of the observation points (-24; -16; -8; -1 h). In Figure 3.4.2 the individual growth rate for each patient is plotted against the MFD at 1 h prior to laparoscopy. The rank correlation coefficient for the entire study population was 0.075. The wide range of individual growth rates (-1.6 to +8.3 mm /24 h) is scattered equally over the monofollicular and multifollicular cycles.

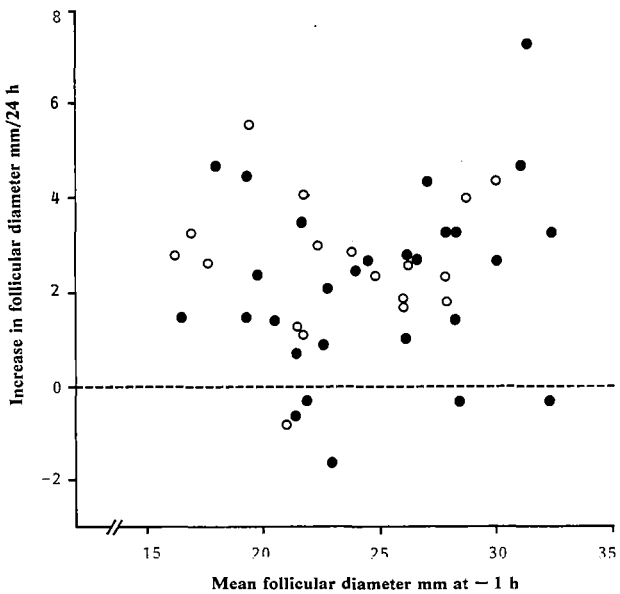

Fig. 3.4.2
The individual follicular growth rate related to follicular size (MFD) at 1 h prior to laparoscopy; ○ monofollicular cycles (n = 18); ● multifollicular cycles (n = 28)

Discussion

Follicular growth profiles in spontaneous and induced cycles show a linear pattern in the late follicular phase with a daily increase of the MFD ranging between 1.5 and 2.7 mm (Hackelöer and Robinson, 1978; Hackelöer et al., 1979; Renaud et al., 1980; O'Herlihy et al., 1980a, 1982b; Kerin et al., 1981; Bryce et al., 1982; Vargyas et al., 1982; Mantzavinos et al., 1983). However, the growth conduct of the dominant follicle during and after the spontaneous LH surge remains controversial. In the majority of the studies a continuous linear growth is established following the LH surge up to the supposed moment of ovulation (Hackelöer and Robinson, 1978; O'Herlihy et al., 1980a, 1982b; Kerin et al., 1981; Bryce et al., 1982; Vargyas et al., 1982; Mantzavinos et al., 1983). Hackelöer et al. (1979) and Honda et al. (1984) found a constant size or even a decrease in MFD during this particular period, whereas Queenan et al. (1980) established an exponential growth pattern. These contradictory findings may be explained by: (a) differences in interval length between subsequent ultrasound observations; (b) differences in definition of ultrasonic signs of ovulation, and (c) differences in patient selection. Little is known about the conduct of the dominant follicle in CC or HMG stimulated cycles following HCG administration. In the present study follicular growth in CC /HCG stimulated cycles during the last 24 h prior to laparoscopic follicle aspiration shows a linear pattern, resulting in a mean increase in MFD over this period of 2.5 mm /24 h, which is not significantly different from the value of 2.2 mm /24 h found in a previous study regarding a 72 h period before laparoscopy (Leerentveld et al., 1985). The administration of HCG does not seem to alter the follicular growth rate as established earlier in the follicular phase.

Finally, the difference in the mean values of the MFD between the monofollicular and multifollicular subgroups in the present study is at variance with a previous report (Leerentveld et al., 1985) in which such a difference could not be established. Although the patient selection criteria were the same in both studies, this discrepancy may be determined by the different patient distribution over the monofollicular and multifollicular cycles. Moreover, in contrast to our earlier study (Leerentveld et al., 1985) in which HCG was administered depending on follicular size, in the present study HCG was given according to a fixed treatment scheme. This may also explain the differences in MFD at 24 and 1 h before laparoscopy between the previous and present study.

3.5 Conclusions

Both in the natural and CC stimulated cycle the daily increase in MFD in the late follicular phase follows a linear pattern up to the moment of supposed ovulation or HCG administration. In CC stimulated cycles the mean growth rate is increased (2.2 mm /24h) when compared with spontaneous cycles (1.6

mm /24h), resulting in significantly larger follicles on days -2, -1 and on day 0, the day of follicle aspiration. This increased growth rate was established both under monofollicular and multifollicular situations. The mean growth rate in stimulated cycles, however, only allows a crude prediction of ultimate preovulatory follicular dimensions, as there is substantial variation in the growth rate of individual follicles.

Concerning follicular growth in the 24 h period preceding follicle aspiration, also here a linear increase in mean follicular diameter (2.5 mm) was found. The administration of HCG does not seem to alter the follicular growth rate established earlier in the follicular phase.

CHAPTER 4

THE SIGNIFICANCE OF PLASMA 17β-OESTRADIOL DETERMINATIONS IN IN-VITRO FERTILIZATION TREATMENT CYCLES

4.1 Literature on the significance of plasma E2 determinations in in-vitro fertilization treatment cycles

Although determinations of urinary or plasma estrogens have been used as a primary (Jones et al., 1983; Mantzavinos et al., 1983) or secondary (Laufer et al., 1983b) monitoring method in stimulated IVF cycles, the role and significance of these measurements with respect to the planning of oocyte retrieval and the prospects of a pregnancy remain controversial, as has been described extensively in Section 1.2.4.

Of interest, therefore, are recent reports on oocyte retrieval in cycles in which no monitoring was employed (Frydman et al, 1986a, 1986b; Wardle et al., 1986; Rainhorn et al., 1987). Pregnancy rates were comparable with those achieved in traditionally monitored patients, although within both the programmed and monitored group in an unacceptably large number of patients no oocytes were obtained during follicle aspiration.

4.2 Introduction to own observations

In view of the conflicting data in the literature on the correlation of plasma E2 values and findings by ultrasound (see also Section 1.2.4), both variables were studied in CC induced cycles and related to treatment outcome (Section 4.3).

Since at a later stage our IVF programme changed from follicular stimulation by CC alone to a HMG superovulation protocol, the need arose to define a normal E2 range and try and correlate this range with treatment outcome. This particular study is presented in Section 4.4.

Many IVF centres base their daily treatment policy on the findings published by Jones et al. (1983). These authors related treatment outcome to E2 profiles as established during the late follicular phase. The highest pregnancy rate was achieved in the presence of the most frequent profile A, in which E2 levels continue to rise following HCG administration. In the presence of the remaining E2 profiles (B-G) substantially lower pregnancy rates were observed. However, these groups with an apparently poor prognosis were relatively small and differences were not statistically significant. Nevertheless, many IVF teams cancel

follicle aspiration when E2 levels drop following HCG administration. To assess the impact of E2 profiles following HCG administration on treatment outcome in a larger series, the study presented in Section 4.5 was undertaken.

4.3 Monitoring of clomiphene citrate stimulation by means of plasma 17β-oestradiol determinations and ultrasonographic follicle measurements in in-vitro fertilization treatment cycles

R.A. Leerentveld,[1] G.H. Zeilmaker,[2] M. van der Stoep,[1] J.W. Wladimiroff,[1] F.H. de Jong,[3] A.Th. Alberda[1]

[1]Department of Obstetrics & Gynaecology, Erasmus University Rotterdam, The Netherlands
[2]Department of Endocrinology, Growth & Reproduction, Erasmus University Rotterdam, The Netherlands
[3]Department of Internal Medicine III, Erasmus University Rotterdam, The Netherlands

Published in Human Reproduction 2:187, 1987
(Reproduced with permission of IRL press Ltd.)

Summary

In 57 IVF cycles stimulated with CC the relationship between plasma E2 and ultrasonographic measurements of follicular diameter was assessed. Under both monofollicular and multifollicular conditions a wide range in plasma E2 values was observed in the late follicular phase. No significant correlation could be established between the dimensions of the dominant preovulatory follicle and plasma E2 values, both in monofollicular and multifollicular cycles. Pregnancies and conceptions occurred in cycles with both low and high circulating E2 levels. In pregnancy cycles a slight increase in plasma E2 values was found on the day following administration of HCG. In conceptional cycles not leading to a clinical pregnancy, plasma E2 profiles varied considerably, whereas in cycles in which no oocytes were fertilized, plateauing or a distinct decrease occurred during this particular period. The present study suggests that the relative daily increase in plasma E2 values may be the most relevant aspect of plasma E2 monitoring.

Introduction

In 1980 Edwards et al. described the treatment procedure of the first IVF cycle leading to the birth of a healthy infant. Graafian follicle development in a spontaneous cycle was monitored by urinary oestrogen determinations and laparoscopic follicle puncture was scheduled by serial assays for urinary LH. A number of studies regarding monitoring follicle growth for IVF in both spontaneous (Kerin et al, 1980; Lopata et al., 1980; Mettler et al., 1982) and anti-oestrogen or gonadotrophin stimulated cycles (Jones et al., 1982, 1983; Laufer et al., 1983b; Lopata et al., 1983) has been published. Currently cervical mucus score, vaginal cytology, urinary excretion of estrogens, plasma E2 determinations and ultrasonographic follicle measurements are employed for monitoring folliculogenesis and planning of HCG administration. A combination of two or more of these variables is used in the majority of IVF programmes.

At present many IVF groups employ combined plasma E2 determinations and ultrasonographic follicle measurements in the assessment of Graafian follicle development. A close correlation between plasma E2 levels and the dimensions of the dominant follicle has been established in spontaneous as well as stimulated cycles by some (Hackelöer et al., 1979; Marrs et al., 1983), but refuted by others (Beck et al., 1981; Mantzavinos et al., 1983). Multiple follicle development could partly account for these contradictory findings (Mantzavinos et al., 1983).

In the present study, the value of plasma E2 determinations in monitoring ovarian response in CC stimulated cycles was assessed under both monofollicular and multifollicular conditions. An attempt was made to elucidate the relationship between plasma E2 profiles and ultrasonographically determined follicular growth under these conditions.

Materials and methods

Forty-nine regularly cycling women with tubal infertility were studied in 57 IVF cycles. All were stimulated with 100 mg CC/day for 5 days, starting on day five of the menstrual cycle in order to induce multifollicular development. Follicular measurements were carried out at 2-day intervals from day nine or ten of the cycle and in 79% of cycles plasma E2 determinations were performed on the same day. When ultrasound revealed a dominant follicle with a MFD > 15 mm, daily scans and plasma E2 determinations were performed. A dose of 5,000 IU HCG was given when ultrasound showed a dominant follicle with an MFD $\geqslant 20$ mm, independent of the number and size of secondary follicles. Laparoscopy was scheduled 35-36 h following HCG administration. In 46% of cycles, the MFD and E2 values were also determined on the day of follicle puncture (day 0). In nine cycles, monofollicular growth occurred, as established during laparoscopy (group I). Aspirates with a volume < 1 ml were disregarded. In twenty cycles two follicles developed (group II), three were identified in 18 cycles (group III), and four or more in the remaining ten cycles (group IV).

All these follicles were previously identified by ultrasound and oocyte recovery was virtually 100 %. Within the 57 treatment cycles, extracorporal fertilization was established in 41 (conceptional cycles) of which four resulted in clinical pregnancies, fertilization did not occur in 15 cycles (non-conceptional cycles), in eight of which oligozoospermia was present (sperm count < 20 million / ml). No oocytes were collected in one treatment cycle.

Blood samples were obtained between 09.00 h and 13.00 h. Plasma E2 levels were estimated by radioimmunoassay, using kits provided by EIR, Würenlingen, Switzerland. Intra-assay and inter-assay variations amounted to 7.4% and 11.0%, respectively.

A Kretz Combison 100 mechanical sector scanner (Kretztechnik GmbH, Zipf, Austria) was used for follicle meaurements, employing a 2.5 MHz transducer. Electronic calipers were used and calibration was set at 1540 m /sec. The MFD was determined as described by us previously (Leerentveld et al., 1984).

Data on E2 levels were not obtained in three cycles, and no ultrasonographic follicle measurements were performed in five cycles, leaving 54 and 52 cycles, respectively, for further analysis. Data on days -5 and -4 were too scarce to allow any conclusion to be drawn, so these observation days were not taken into account.

A mean MFD (mm) $\pm$ 1 S.D. on day 0 was calculated for groups I-IV from a total of 41 conceptional cycles, for comparison with single MFD values in seven non-conceptional cycles in the presence of normal sperm counts. The remaining 8 non-conceptional cycles with oligozoospermia were disregarded.

The Mann-Whitney two-sample rank sum test was employed for comparison of the individual E2 values between the four groups for each observation day. The correlation between individual MFD values of the dominant follicles and corresponding plasma E2 values within these four groups for each observation day, was assessed using Spearman's rank correlation test (Table 4.3.1).

Table 4.3.1
Correlation between individual mean follicular diameters of the follicle and corresponding plasma E2 values. Data given for all four study groups on each observation day (day 0 = day of follicle puncture), using Spearman's rank correlation test.

Group		Day -3	Day -2	Day -1	Day 0
I	r =	+ 0.80*	+ 0.16*	+ 0.03*	+ 0.36*
II	r =	− 0.15*	+ 0.22*	− 0.05*	+ 0.70*
III	r =	+ 0.29*	0.00*	0.00*	− 0.50*
IV	r =	+ 0.97*	− 0.70*	− 0.66*	+ 0.81**

* Not significant ($P > 0.05$).
** Significant ($P < 0.05$).

Results

Figure 4.3.1 shows individual plasma E2 values on days -3, -2, -1 and 0 for each of the four study groups. Different symbols were given to the values representing pregnancy cycles (n = 4), conceptional cycles not resulting in clinical pregnancies (n = 37), non-conceptional cycles with normal (n = 7) and reduced sperm count (n = 8) and the one treatment cycle in which no oocytes were collected. Additionally the E2 profiles for the four pregnancy cycles are presented.

On two occasions, extra-corporal fertilization was accomplished using oligozoospermic semen (8 and 15 million spermatozoa /ml in group I and III, respectively). Both under monofollicular and multifollicular conditions a wide range in plasma E2 values was observed on days -3 to -1. Differences in plasma E2 levels between the four study groups were virtually absent and were statistically

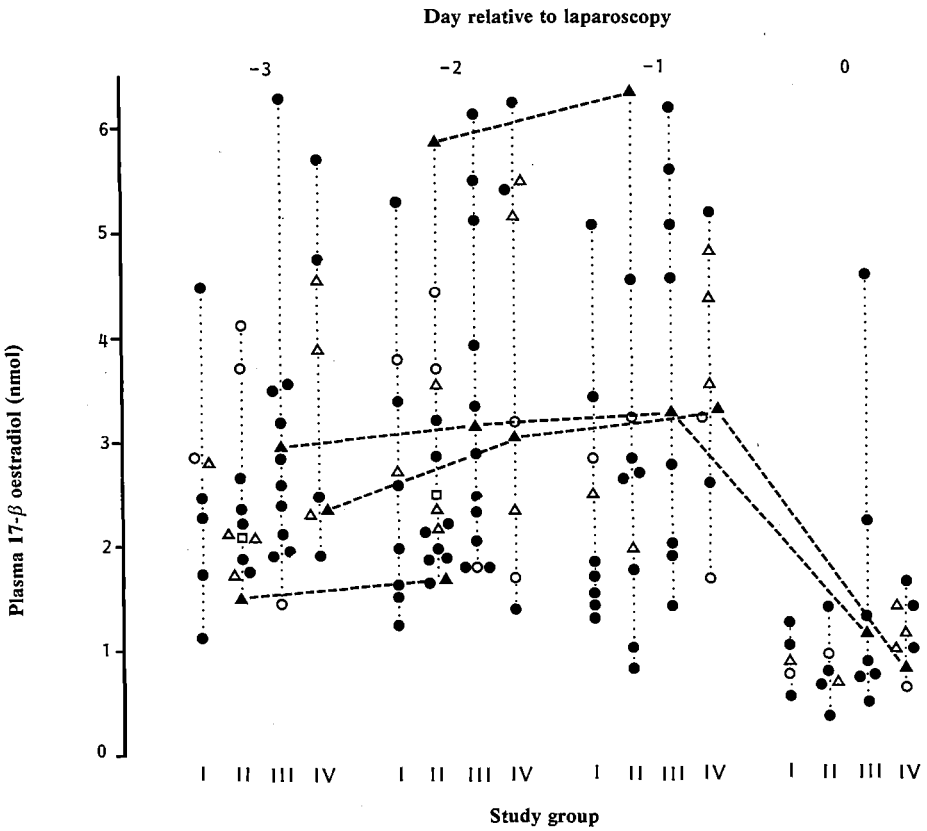

Fig. 4.3.1
Individual oestradiol levels for each observation day in the separate study groups; ▲ pregnancy cycles; ● conceptional cycles; ○ non-conceptional cycles; △ non-conceptional cycles in the presence of oligozoospermia; □ no oocytes collected; ------plasma E2 profiles in pregnancy cycles.

insignificant. Except for one cycle with three follicles a decrease in plasma E2 levels was noticed between days -1 and 0 in every treatment cycle. This decrease was most marked in those cycles showing E2 values exceeding a level of 3,000 pmol /l on day -1. No significant correlation between individual plasma E2 values and the MFD of the dominant follicle was established for all four study groups for each observation day, except on day 0 in group IV ($P < 0.05$).

In Figure 4.3.2 individual MFD values for each study group on day 0 from the non-conceptional cycles with normal sperm count were plotted against the mean MFD ± 1 S.D. on day 0 from the 41 conceptional cycles. All MFD values from the cycles in which no fertilization was observed were situated within ± 1 S.D. of the mean MFD established in cycles leading to fertilization, apart from one value of 21.7 mm in study group III.

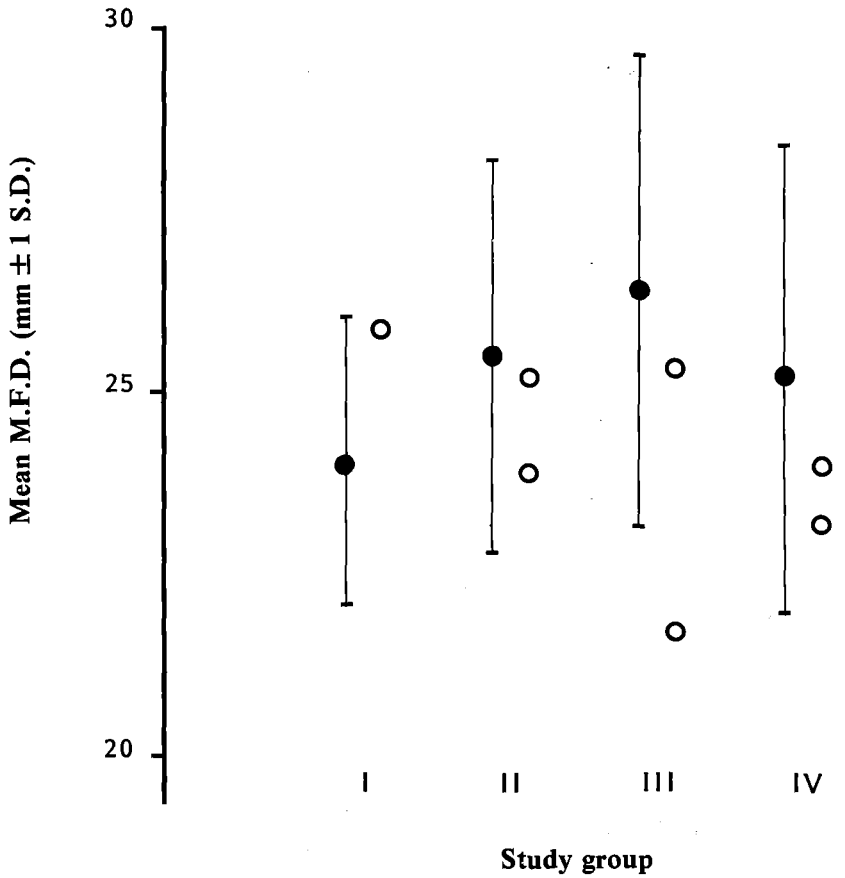

Fig. 4.3.2
Dimensions of the dominant follicle on day 0 in seven nonconceptional cycles in the presence of normal sperm counts (○) relative to the mean size of the dominant follicle in 41 conceptional cycles (●).

Discussion

In the present study, a wide range in plasma E2 values during the late follicular phase can be demonstrated in cycle stimulated with CC whether one or more follicles are developing, suggesting a lack of correlation between E2 levels and number of follicles exceeding a volume of 1 ml. Although E2 values tend to be higher in multifollicular cycles, there were no significant differences between the four study groups on all days of investigation. Conceptions and pregnancies occurred in study groups II, III and IV, in cycles with both low and high circulating E2 levels, confirming the findings of Smith et al., 1983. It appears, therefore, that peak plasma E2 values cannot serve as a reliable predictor for the occurence of fertilization of the collected oocytes. However, close observation of E2 profiles during three cycles in which clinical pregnancies did occur, shows a slight increase in plasma E2 levels between days -2 and -1 (4.5, 8.6 and 8.7 %, respectively). In one pregnancy cycle the plasma concentration of E2 on day -1 was not available. Although these relative increases remain within the range of the inter-assay variation, the E2 profiles closely follow "pattern A" and "group A" in HMG stimulated cycles as described by Jones et al. (1983) and Laufer et al. (1986) respectively. These profiles were associated with the highest pregnancy rate (27 % and 17 %) and are characterized by a daily increase in plasma E2 levels up to the day preceding follicle aspiration. The concentration of E2 had usually fallen on the day of follicle puncture.

Although in the present study all three pregnancies under investigation are associated with pattern A /group A values, the number is too small for statistical analysis. In conceptional cycles not leading to pregnancy an increase as well as a decrease in plasma E2 levels was observed between days -2 and -1. In all non-conceptional cycles in the presence of normal sperm counts, plateauing or a decrease in E2 values by up to 27 % was demonstrated during this particular period, resembling "patterns B-E" according to Jones et al. (1983) and "group B" as described by Laufer et al. (1986). A marked decrease in E2 levels was found in all but one cycle (group III) during the 24 h preceding follicular aspiration, resulting in a rather narrow range in E2 values on day 0 for groups I, II and IV.

Discrepancy exists in the literature concerning the correlation between circulating E2 levels and the dimensions of the dominant follicle as established by ultrasound in spontaneous single-follicle cycles. Whereas some authors established a very close correlation in such cycles (Hackelöer et al., 1979), or even in stimulated cycles with several follicles (Marrs et al., 1983) , others found a poor relationship in spontaneous single-follicle cycles (Beck et al., 1981; Bryce et al., 1982), as well as in stimulated cycles with several follicles (Mantzavinos et al., 1983). Data from the cycles with a single follicle (group I) in the present study, are in agreement with the study of Beck et al. (1981). In cycles with several follicles there was virtually no correlation between E2 levels and the dimensions of the leading follicle as determined by ultrasound (Cabeau and Bessis, 1981; Schmidt et al., 1981; Jones et al., 1983; Mantzavinos et al., 1983).

This finding was confirmed in the present study as the relation between the MFD of the dominant follicle and E2 levels in groups II to IV was rather poor. An explanation for these contradictory findings has not yet been provided.

Follicular dimensions at a time close to ovulation did not show any significant difference between conceptional and non-conceptional cycles. As a result, determination of the preovulatory MFD value of the dominant follicle is not suitable as a predictor for the occurence of fertilization in vitro of the oocytes obtained. As the relative daily increase in plasma E2 levels in the late follicular phase seems to be the most relevant aspect of plasma E2 monitoring, the combined use of ultrasound and plasma E2 determinations is imperative for monitoring IVF patients. As in the present study, no baseline plasma E2 levels were determined, no comparison could be made with regard to the findings of McBain et al. (1985), Levran et al. (1985) and Quigley et al. (1985) that the best rates of fertilization and pregnancy are accomplished following HCG administration after six days of E2 rise.

4.4 The value and role of plasma 17β-oestradiol measurements during ovarian hyperstimulation for in-vitro fertilization

R.A. Leerentveld,[1] H.A.B. Janssen-Caspers,[1] H.C. van Os,[1] M.C.W. Scholtes,[1] J.W. Wladimiroff,[1] G.H. Zeilmaker,[2] F.H. de Jong,[3] A.Th. Alberda[1]

[1]Department of Obstetrics & Gynaecology, Erasmus University Rotterdam, The Netherlands
[2]Department of Endocrinology, Growth & Reproduction, Erasmus University Rotterdam, The Netherlands
[3]Department of Internal Medicine III, Erasmus University Rotterdam, The Netherlands

(Accepted for publication in Human Reproduction)

Summary

Follicle aspiration was performed in 464 hyperstimulated IVF treatment cycles in patients with severe tubal damage as the sole cause of their infertility. In 413 cycles 1-4 embryos could be replaced, resulting in 102 clinical pregnancies. In 458 treatment cycles, data on plasma E2 levels were available on days -3 and -2, in 322 cycles also on days -1 and 0, day 0 being the day of follicle puncture. Although the distribution of cycles leading to clinical pregnancy within the 5-95th centile for plasma E2 levels differed from that observed outside this range, these differences were of no statistical significance. Our results indicate that IVF pregnancies occur in the presence of a wide range of E2 levels during the 3 days period preceding follicle aspiration. The importance of plasma E2 measurements for treatment policy, therefore must be reconsidered.

Introduction

At present the majority of IVF teams employ a combination of ultrasonographic follicle measurements and plasma or urinary oestrogen determinations for monitoring both morphological and functional aspects of folliculogenesis. Some groups claim a close correlation between both variables even in stimulated multifollicular cycles (Marrs et al., 1983). Other authors could not establish such a relationship (Mantzavinos et al., 1983; Hünlich et al., 1984; Friedrich et al., 1986).

Studies on plasma E2 levels during IVF hyperstimulated cycles have mainly focused on E2 response in terms of absolute preovulatory E2 levels (Garcia et al., 1983; Jones et al., 1983; Dlugi et al., 1984; Laufer et al., 1986; Zarutskie et al., 1987; Forman et al., 1988), E2 profiles (Jones et al., 1983; Diamond et al., 1985; Dor et al., 1986; Laufer et al., 1986; Leerentveld et al., 1987) and the duration of E2 rise above baseline values preceding follicle aspiration (Levran et al., 1985; Quigley et al., 1985). Pregnancy rates seemed to be higher at high preovulatory E2 levels (high responders) in several studies (Garcia et al., 1983; Jones et al., 1983; Laufer et al., 1986). This is at variance with the findings of Forman et al. (1988), who reported decreased pregnancy rates at high preovulatory E2 levels following the transfer of one or two embryos. This difference was not observed after transfer of three embryos. Increasing E2 levels preceding and following HCG administration (Jones et al., 1983; Diamond et al., 1985; Dor et al., 1986; Laufer et al., 1986; Leerentveld et al., 1987) seemed to be associated with a higher pregnancy rate. Moreover, a sustained E2 rise of six days duration above baseline values preceding the moment of HCG administration was associated with a more favourable pregnancy rate as compared with that observed after a shorter or longer period of E2 rise (Levran et al., 1985; Quigley et al., 1985).

In the majority of these studies similar trends could be detected, but the numbers of treatment cycles, in particular in the groups with an unfavourable prognosis, were too small to allow statistically significant conclusions.

Recently Okamoto et al. (1986) defined a range of plasma E2 concentrations during a treatment period of eight days in 102 cycles leading to pregnancy after CC /HMG stimulation. However, no comparison was made with the plasma E2 range of treatment cycles in which no pregnancy was established.

In the present study the distribution of plasma E2 values in the three day period preceding follicle aspiration is presented. Employing the individual E2 values found in pregnancy cycles, an attempt has been made to define a reference E2 range in which treatment prognosis might be favourable.

Materials and methods

In our IVF programme 610 treatment cycles were started from 1st January 1986 to 30th September 1987 in patients up to the age of 40 with tubal pathology

as the only known cause of infertility in the presence of normal semen parameters ($\geqslant 20.10^6$ motile spermatozoa /ml). A total of 146 cycles was cancelled (Table 4.4.1), leaving 464 cycles in which follicle aspiration was performed. These cycles resulted in 102 clinical pregnancies (22.0 % positive pregnancy test). Six of the initial 610 treatment cycles were excluded from the study, as no data on plasma E2 values were available on day -3, the day of follicle aspiration being day 0, resulting in 604 treatment cycles and 458 follicle punctures for further analysis. In these 458 cycles ovarian hyperstimulation was induced with HMG only (N = 413), a combination of CC and HMG (N = 28) or HMG plus FSH (N = 7), starting on day 2, 3 or 4 of the menstrual cycle, depending on previous cycle length and the type of stimulation. In 10 cycles HMG administration was commenced during GnRH analogue therapy. The mean age of the women was 32.9 years with a range from 23 to 39 years. Monitoring of ovarian response by means of ultrasound and plasma E2 determinations was started on day 6, 7 or 8 of the menstrual cycle.

Table 4.4.1
Reasons for cancellation and numbers of cancelled cycles.

Main reason for cancellation	Number of cycles
E2 levels < 1400 pmol/l in the presence of a largest MFD ≥ 18mm	13
Decreasing (> 10 %/24 h) E2 levels with a largest MFD < 17 mm	22
Dominance (second follicle MFD < 15 mm)	26
Insufficient follicular growth as judged during ultrasonography	32
Presence of ovarian cysts precluding adequate ultrasonography	16
Premature ovulation	28
Miscellaneous	9
Total number of cancelled cycles	146

Follicular measurements were partly carried out transabdominally as described previously (Leerentveld et al., 1984), employing a Kretz Combison 320 mechanical sector scanner (Kretztechnik GmbH, Zipf, Austria) with a 3.5 MHz

transducer. The majority of the follicular measurements, however, was performed transvaginally, using a 5 MHz vaginal probe. Also here, an MFD was calculated from the maximal diameters in three perpendicular planes.

Blood samples were drawn between 09.00 a.m. and 01.00 p.m. Plasma E2 levels were measured by rapid radioimmunoassay, employing kits provided by Diagnostic Products Corporation, Los Angeles, California, U.S.A. The intra-assay variation was up to 7.3 %, the inter-assay variation amounted to 8.7 %.

Depending on the individual ovarian response as judged by ultrasound and plasma E2 values, the usual daily dosage of 225 IU of HMG was increased to 300 IU in only very few cases. In the presence of at least two follicles with a MFD exceeding 15 mm, 10,000 IU of HCG were administered when one of the following conditions was met: (i) MFD of the dominant follicle $\geqslant$ 17 mm and plasma E2 level $\geqslant$ 1,800 pmol/l; or (ii) MFD of the dominant follicle $\geqslant$ 18 mm and plasma E2 level 1,400- 1,800 pmol/l. When plasma E2 levels were below 1,400 pmol/l the cycle was usually abandoned, except when on basis of previous experience, a plasma E2 level meeting our criteria was not to be expected in a particular woman. When prior to HCG administration a drop in plasma E2 level exceeding 10 % of that of the previous day was observed, the cycle was also passed. In 448 cycles ultrasonically guided transvaginal follicle puncture was performed as an out-patient procedure under local analgesia 35 h following HCG administration. Six patients underwent percutaneous transvesical puncture and in four patients follicle aspiration was done during laparoscopy.

In all 458 cycles under study plasma E2 values on days -3 and -2 were available. Since E2 values on days -1 and 0 were only determined as from the 1st of September 1986, 322 treatment cycles, resulting in 74 pregnancies, were available for data analysis for these days.

Detailed information on the IVF laboratory procedures and on the embryo transfer technique has been published elsewhere (Zeilmaker, 1986). At present 7.5 % heat inactivated patient's serum is used in the culture and transfer medium.

Statistical analysis of differences in distribution of pregnancies was performed using Fisher's exact test for each observation day separately. The differences in oocyte yield and replaced embryos were analyzed employing Wilcoxon's test.

Results

In Table 4.4.2 the outcome in the 458 cycles under study is presented. In 413 cycles 1-4 (mean 3.0) embryos could be transferred into the uterine cavity, resulting in 102 clinical pregnancies (pregnancy rate 22.0 % per follicle puncture). Twenty-four patients had a first trimester abortion and one patient had an ectopic pregnancy, resulting in an ongoing pregnancy rate of 16.6 % per follicle aspiration.

In Figure 4.4.1 data on plasma E2 values are presented as the range and the 5th, 50th, and 95th centiles per observation day. Individual plasma E2 levels in pregnancy cycles are plotted in the E2 distribution of the total population under study. Different symbols are used to indicate the E2 values of cycles

leading to spontaneous abortion, ectopic pregnancy or ongoing pregnancy. Pregnancy cycles ending in spontaneous abortion occurred both at low and high circulating E2 levels and were equally distributed over the three E2 ranges on all observation days.

Table 4.4.3 displays the oocyte yield for the three ranges (< 5%, 5-95%, > 95%). For each observation day a statistically highly significant difference in oocyte harvest was established between the three E2 ranges.

The mean number of replaced embryos per individual in the E2 range below the 5th centile was significantly lower, when compared with that observed at E2 levels exceeding the 95th centile (Table 4.4.4).

In Table 4.4.5 the pregnancy rates per E2 range per observation day are presented. The distribution of pregnancy cycles within the 5-95th centile range was different from that outside this range. However, this difference was not statistically significant on any observation day.

Table 4.4.2
Outcome of 458 treatment cycles under study

Treatment cycles	610	
Total number of follicle punctures	464	
Cycles in which ≥ 1 oocytes obtained	463	(99.8 %)
Study population:		
Follicle punctures, E2 values available on days − 3 and − 2	458	
Follicle punctures, E2 values also known on days − 1 and 0	322	
Number of embryo transfers	413/464	(90.2 %)
Pregnancies	102/464	(22.0 %)
First trimester abortions (including one ectopic pregnancy)	25/464	(5.4 %)
Ongoing pregnancies	77/464	(16.6 %)

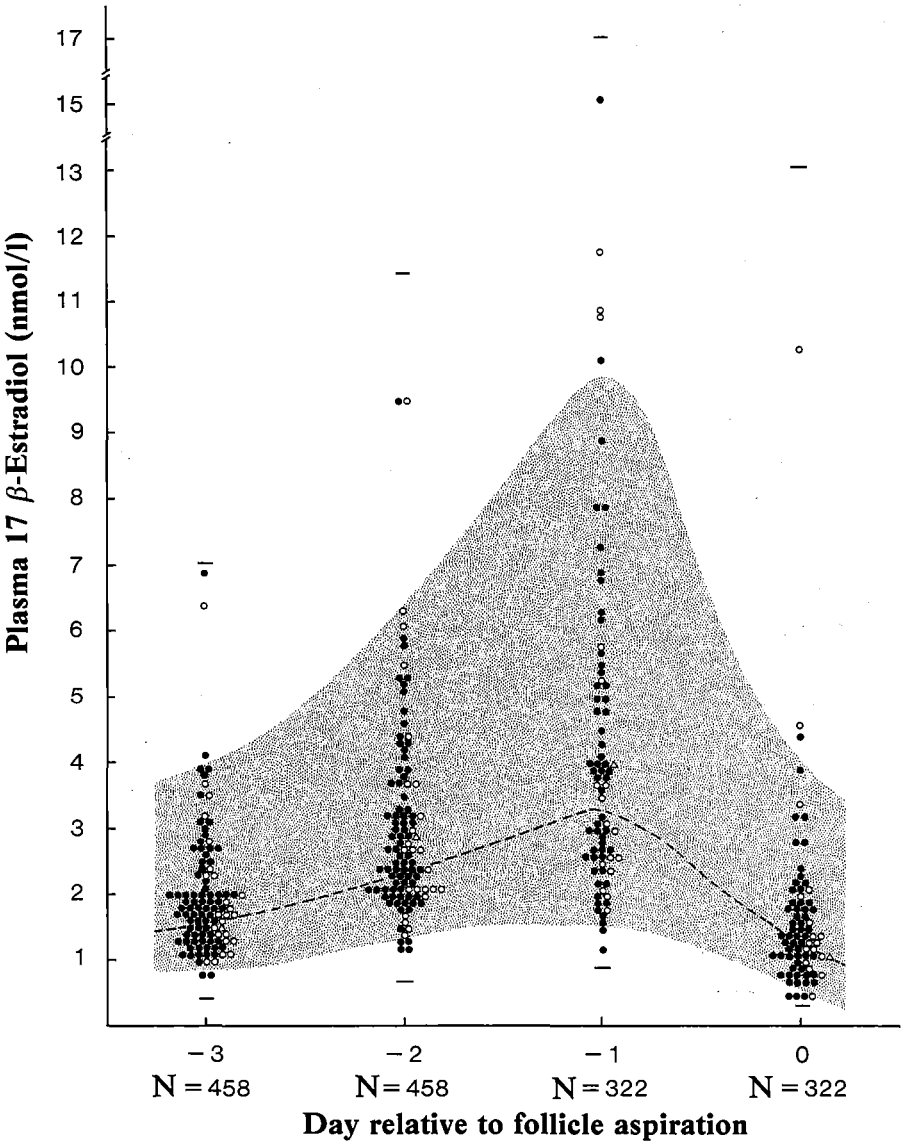

Fig. 4.4.1
Distribution of plasma 17β-Oestradiol values of the total studygroup (N = 458 on days − 3 and − 2; N = 322 on days − 1 and 0); ▒ 5th - 95 th centile; ------ 50th centile; —— range. Individual E2 values are given for ongoing pregnancies (●), spontaneous abortions (○) and an ectopic pregnancy (△).

Table 4.4.3
Mean oocyte yield per E2 centile range for each observation day relative to follicle aspiration (day 0)

	Observation days			
	− 3[a]	− 2[b]	− 1[c]	0[d]
< 5th centile	3.2	3.3	3.8	3.4
≥ 5th − ≤ 95th centile	6.5	6.5	7.1	7.3
> 95th centile	17.0	17.0	17.4	13.9
Total number of cycles	458	458	322	322

a,b,c,d, $P < 1.10^{-5}$

Table 4.4.4
Mean number of embryos per transfer in the < 5th centile and > 95th E2 centile ranges for each observation day relative to follicle aspiration (day 0)

	Observation days			
	− 3[a]	− 2[b]	− 1[c]	0[d]
< 5th centile	2.1	2.1	2.3	2.3
> 95th centile	3.6	3.4	3.2	3.0

a,b $P < 1.10^{-6}$
c $P < 1.10^{-2}$
d $P < 5.10^{-2}$

Discussion

From Figure 4.4.1 it can be seen that pregnancies as well as spontaneous abortions are observed both in the presence of low and high circulating E2 levels. Pregnancy rates outside the 5th-95th centile are reduced when compared with the rates within this range. This trend was noticed on all observation days preceding follicle aspiration except below the 5th centile on day -1. The differences

Table 4.4.5
Pregnancy rate (pregnancies per 100 follicle punctures) per E2 range (< 5th centile; ≥ 5 th - ≤ 95th centile; > 95th centile) per observation day. Number of cycles on days − 3 and − 2: 458; number of cycles on days − 1 and 0: 322

	Observation days			
	− 3[a]	− 2[b]	− 1[c]	0[d]
≥ 5th − ≤ 95th centile	23.5 (97/412)	23.3 (96/412)	23.1 (67/290)	23.1 (67/290)
< 5th centile	8.6 (2/ 23)	17.4 (4/ 23)	12.5 (2/ 16)	25.0 (4/ 16)
> 95th centile	13.0 (3/ 23)	8.6 (2/ 23)	31.2 (5/ 16)	18.8 (3/ 16)
Total numbers of cycles	458	458	322	322

[a,b,c,d] N.S. ($P > 5.10^{-2}$)

however, were not statistically significant, and thus may be attributed to chance. Similar findings were recently reported by Forman et al. (1988).

The significant reduction in the mean oocyte yield and the mean number of replaced embryos in the low E2 range as compared with the > 95th centile range is not reflected in a lower pregnancy rate. At the same time, the significantly increased oocyte harvest in the high E2 range, allowing us to replace a significantly higher number of selected embryos, does apparently not result in a higher pregnancy rate.

The majority of pregnancies obtained in cycles in the low E2 range was a result of multiple embryo transfer. The chance on a pregnancy in the 5th-95th centile range was closely correlated with the number of transferred embryo's, confirming the findings of Edwards et al. (1984), Jones et al. (1984) and Testart et al. (1985). This correlation was not found in cycles above the 95th centile for E2.

At low plasma E2 levels and in the 5th-95th centile range the prospect of a pregnancy therefore seem mainly determined by the number and quality of replaced embryos.

With respect to the high number of oocytes obtained at plasma E2 levels exceeding the 95th centile, resulting in a very high number of multiple embryo transfers, one might expect a higher pregnancy rate in this particular E2 range. This, however, is not observed. In our opinion high circulating E2 levels may have a negative impact on endometrial receptivity, as has been also suggested by Friedrich et al. (1986) and Forman et al. (1988). This view seems to be supported by our observation that one patient responding with E2 levels above the 95th centile, did conceive in a subsequent natural cycle as a result of the transplantation of thawed embryos obtained in the preceding treatment cycle. The pregnancy rate in this high E2 range could also have been influenced by disrupted luteal function related to the high amounts of circulating oestrogens, as suggested by Messinis et al. (1987).

The retrospectively defined 5th-95th range in our entire study population is similar to the prospectively designed range derived from pregnancy cycles as published by Okamoto et al. (1986). In their curve, however, the narrowing of E2 concentrations at midcycle is absent, probably as a result of asynchrony of follicular development in the study cycles, a consequence of a prospective analysis. Furthermore, in the study of Okamoto et al. (1986) the entire E2 range is shifted to a higher level when compared with the present reference curve. This may be the result of differences in treatment regimens, in criteria for HCG administration, and possibly of different E2 assay procedures. As these authors do not present any data on E2 concentrations in cycles not resulting in pregnancy, a comparison with respect to their entire study population cannot be made. From these apparently conflicting findings it may be concluded that all variables mentioned should be taken into account when comparing E2 reference ranges.

From the present study it may be concluded that IVF pregnancies not only occur in the presence of both low and high plasma E2 levels, but also that

pregnancy cycles are equally distributed over the entire plasma E2 range. The importance of E2 determinations for daily treatment policy with respect to the moment of HCG administration must therefore be reconsidered. This conclusion is supported by the study of Rainhorn et al. (1987), in which a similar pregnancy rate was established in programmed oocyte retrieval cycles without any monitoring, when compared with IVF cycles monitored by plasma E2 determinations and ultrasonographic follicular measurements.

4.5 Significance of plasma 17β-oestradiol profiles following the administration of human chorionic gonadotrophin in in-vitro fertilization treatment cycles

R.A. Leerentveld,[1] A.Th. Alberda,[1] H.A.B. Janssen-Caspers,[1] H.C. van Os,[1] M.C.W. Scholtes,[1] J.W. Wladimiroff,[1] G.H. Zeilmaker,[2] F.H. de Jong[3]

[1]Department of Obstetrics & Gynaecology, Erasmus University Rotterdam, The Netherlands
[2]Department of Endocrinology, Growth & Reproduction, Erasmus University Rotterdam, The Netherlands
[3]Department of Internal Medicine III, Erasmus University Rotterdam, The Netherlands

(Submitted for publication)

Summary

In 328 hyperstimulated IVF cycles leading to follicle aspiration plasma E2 profiles following the administration of HCG were related to treatment outcome. Seventy five clinical pregnancies were established, resulting in an over all pregnancy rate of 22.9 % per puncture. In the presence of the most common E2 profile (profile A; an increase $>$ 10 % when compared with the E2 value before HCG administration) a pregnancy rate of 22.5 % was found, whereas a decrease in E2 values exceeding 10 % (profile C) was related to a pregnancy rate of 21.6 %. Plateauing E2 values during this particular period (E2 changes remaining within 10 % of the pre HCG value; profile B) were associated with a pregnancy rate of 25.9 %. These differences in pregnancy rates were not statistically significant, indicating that E2 profiles following HCG administration are of no clinical importance.

Introduction

In a previous study regarding plasma E2 profiles in CC stimulated IVF cycles (Leerentveld et al., 1987) it was demonstrated that pregnancy cycles were

associated with a slight increase in plasma E2 values on the day following administration of HCG. In treatment cycles with ET but not resulting in clinical pregnancy, plasma E2 profiles varied considerably, whereas in cycles in which no oocytes were fertilized, plateauing or a distinct decrease occurred during this particular period.

Similar findings were reported by Jones et al. (1983), Ben Rafael et al. (1986), Dor et al. (1986) and Laufer et al. (1986). Plateauing or increasing E2 levels during this particular period were associated with the highest pregnancy rates, whereas decreasing E2 levels indicated a poor treatment outcome. However, the numbers of patients, especially in the groups with an unfavourable prognosis, were too small to allow statistically firm conclusions. Nevertheless, in many IVF centres daily treatment policy is based on these data.

The present study provides data on plasma E2 profiles during the 24 h period following the administration of HCG in a larger series. The pregnancy prospects associated with various plasma E2 profiles are analyzed.

Materials and methods

From the 1st October 1986 until the 30th September 1987 a total of 328 IVF treatment cycles leading to follicle puncture was studied in 188 patients. Severe bilateral tubal disease was the single indication for IVF in this study group. All patients were below the age of forty. In all instances normospermia ($\geqslant 20.10^6$ motile spermatozoa /ml) was established during the laboratory phase.

In 292 cycles ovarian hyperstimulation was induced with HMG (Pergonal, Serono, Haarlem, The Netherlands) alone. The remaining 36 cycles were stimulated with a combination of CC (Clomid, Gist-brocades, Delft, The Netherlands) and HMG (N = 23) or with FSH (Metrodin, Serono, Haarlem, The Netherlands) and HMG (N = 3). In ten cycles HMG stimulating therapy was commenced under Buserelin (Hoechst AG, Frankfurt am Main, FRG) treatment, starting on day 23 of the previous cycle or on day 2 of the treatment cycle. The mean age in the study group was 32.7 years (range 23-39 years).

Treatment was started on day 2, 3 or 4 of the menstrual cycle, depending on previous cycle length and the hyperstimulation protocol.

Ultrasonographic monitoring of follicular growth and plasma E2 measurements was started on cycle day 6, 7 or 8. Follicle measurements were performed transvaginally, using a Kretz Combison 320 mechanical sector scanner (Kretztechnik GmbH, Zipf, Austria) with a 5.0 MHz probe with a 240° sector angle. An MFD was calculated from three maximal diameters at right angles to each other.

Blood samples were taken between 09.00 a.m. and 01.00 p.m. Plasma E2 levels were determined by rapid radioimmunoassay, using kits provided by DPC, Los Angeles, California, USA. The intra-assay and inter-assay variation were 7.3 % and 8.7 % respectively.

The reasons for adjusting the dosage of HMG, the indications for the administration of HCG (Pregnyl, Organon Nederland BV, Oss, The Netherlands) and the motives for cancelling a particular treatment cycle were described in detail elsewhere (Leerentveld et al., 1988).

In 326 study cycles an ultrasonically guided vaginal follicle puncture under local analgesia was performed 35 h following HCG administration as described recently (Janssen-Caspers et al., 1988). In the remaining two cycles a percutaneous transabdominal or a laparoscopic follicle puncture was done.

As in six cycles no data on plasma E2 values on day -1 were available, day 0 being the day of follicle aspiration, a total of 322 cycles was left for further evaluation. In these six excluded cycles one pregnancy was obtained.

The IVF laboratory procedures and the embryo transfer technique have been extensively described elsewhere (Zeilmaker, 1986). At present 7.5 % heat inactivated patient's serum is used in the culture and transfer medium.

Statistical analysis of differences in treatment outcome was performed employing the Chi Square test.

Results

An increase in plasma E2 levels exceeding 10 percent during the day following the administration of HCG (profile A) was found in 227 cycles. In 58 cycles plateauing (profile B; plasma E2 levels remaining within 10 % of the value of the previous day) and in 37 cycles a decrease exceeding 10 percent (profile C) was observed.

In Table 4.5.1 the pregnancy rates as well as the absolute figures for the three subgroups under study are presented. The distribution of pregnancy cycles over these subgroups did not show statistically significant differences, although the highest pregnancy rate was associated with plateauing plasma E2 levels (profile B).

Table 4.5.1
Pregnancy rates for each study group (A: E2 increase > 10 % following HCG administration; B: E2 levels plateauing; C: E2 decrease > 10 % (P = 0.84; N.S.)

	Pregnancy rate per puncture	Pregnancies	Cycles
Profile A:	22.5 %	51	227
Profile B:	25.9 %	15	58
Profile C:	21.6 %	8	37
Totals	23.0 %	74	322

Discussion

Studies on plasma E2 monitoring in IVF treatment cycles have mainly been focused on plasma E2 response (absolute E2 levels) (Garcia et al., 1983; Jones et al., 1983; Dlugi et al., 1984; Laufer et al., 1986 ; Zarutskie et al., 1987; Leerentveld et al., 1988), E2 profiles following HCG administration (Jones et al., 1983; Dor et al., 1986; Laufer et al., 1986; Leerentveld et al., 1987) and the duration of plasma E2 rise above baseline values preceding HCG administration (McBain et al., 1985; Levran et al., 1985; Quigley et al., 1985).

In the presence of high preovulatory plasma E2 levels (high responders), a higher pregnancy rate was established than at low plasma E2 values (low responders) (Garcia et al., 1983; Jones et al., 1983; Laufer et al., 1986). Analysis of the data derived from these studies, however, does not show statistically significant differences, possibly as a result of the low numbers of patients in the subgroups with a poor prognosis. Recently we were able to demonstrate that pregnancies occur in the presence of both high and low circulating E2 levels and that the E2 values in pregnancy cycles are equally distributed over the entire E2 range (Leerentveld et al., 1988).

Studies on plasma E2 profiles following the administration of HCG suggest a better treatment prognosis in the presence of increasing or plateauing E2 levels, as compared with decreasing E2 values (Jones et al., 1983; Ben Rafael et al., 1986; Dor et al., 1986; Laufer et al., 1986). However, also here, our analysis of the available data in the literature does not show any significant difference.

Although in the present study profile B was associated with the highest pregnancy rate, the outcome in all subgroups was essentialy the same. It is concluded therefore that treatment outcome is not influenced by the relative change in plasma E2 levels following HCG administration. As a consequence, monitoring of plasma E2 levels following the administration of HCG is of little or no clinical importance. The cancellation of treatment cycles associated with decreasing E2 levels following the administration of HCG, which is at present treatment policy in many IVF centres, does not seem to be justified.

4.6 Conclusions on E2 monitoring of IVF cycles.

In Section 4.3 it was concluded in a rather small patient population, that the relative daily increase in plasma E2 values might be the most relevant aspect of plasma E2 monitoring in IVF cycles. In four pregnancy cycles, achieved following 57 follicle punctures, an increase in plasma E2 values was observed during days -3 to -1, day 0 being the day of follicle aspiration. This confirmed the findings of Jones et al. (1983), Dor et al. (1986) and Laufer and coworkers (1986), who all found favourable treatment results in the presence of rising E2 levels.

From the study given in Section 4.4 is may be concluded that the prognostic value of a given E2 level with regard to pregnancy prospects is extremely low.

Pregnancies were observed over a very large E2 range during days -3 to day -1, day 0 being the day of follicle aspiration. Even at both extremely low and high estrogen levels pregnancies did occur, and pregnancy rates did not differ significantly when compared with those observed in the normal E2 range. This would imply that single E2 determinations are of little or no value for monitoring of IVF cycles. One restriction should be made: since E2 values were plotted relative to the day of follicle puncture, those patients not attaining a follicle puncture as a result of low oestrogens, as defined in Section 4.4, or as a result of a decline in E2 value exceding 10 % preceding HCG administration, were not taken into consideration. This group of cancelled cycles amounted to 5.7 % of the number of started treatment cycles during the observation period given in Section 4.4. Possibly the very low incidence of cycles in which no oocytes were obtained (2 cycles = 0.4 % per puncture) was attributed to the cancellation of cycles exhibiting very low plasma E2 levels. Therefore, at present, plasma E2 determinations are maintained as a secondary monitoring method in our programme.

In contrast to earlier findings (Leerentveld et al., 1987; Section 4.3) the most favourable treatment results in our more extentive series described in Section 4.5 were obtained when plasma E2 levels were plateauing following HCG administration, although pregnancy rates did not differ significantly with respect to the various E2 profiles. Careful examination of pregnancy cycles in Section 4.3 shows that E2 profiles in these cycles in fact meet the criteria for plateauing profiles as used in Section 4.5. The cancellation of treatment cycles showing a decline in plasma E2 values exceeding 10 % following the administration of HCG, which is current treatment policy in many IVF centres, seems therefore not justified. Moreover, the different pregnany rates in the presence of various profiles reported until now can very well be attributed to chance. As a consequence E2 profiles following HCG administration are no longer determined in our IVF programme.

Our data cannot be compared with those from Levran et al. (1985), McBain et al. (1985) and Quigley et al. (1985), who achieved highest pregnancy rates following a continuous rise of plasma E2 values above baseline levels of six days duration. This is determined by the fact that in the Dijkzigt IVF programme ultrasonographic and endocrine monitoring is only commenced on the 7th or 8th day of the cycle, depending on the previous cycle length. Generally, only E2 values of the three to five days period preceding follicle puncture are available for analysis, and therefore no baseline E2 value can be determined.

CHAPTER 5

CONCLUSIONS

In the first part of this thesis the accuracy and reproducibility of static B-compound and real-time sector ultrasonographic equipment in the observation of Graafian follicles was assessed using in-vivo experiments. From these experiments it may be concluded that both techniques provide accurate information on the presence and number of Graafian follicles, their localization, growth rate and ultimate preovulatory dimensions. The intra-observer variation using static B-compound equipment showed a small pooled standard deviation, indicating a high degree of intra-observer reproducibility. The pooled standard deviation for the inter-observer variation was considerably larger. Therefore, follicular growth should preferably be evaluated by one investigator.

Initially follicle measurements by real-time sector scanners were thought to be less accurate when compared with static B-compound scanning techniques. Our in-vivo study comparing both techniques, showed a slightly higher accuracy employing the latter, although differences might have been attributed to chance.

In the second part of this thesis Graafian follicular growth was assessed, both in the natural and CC stimulated cycle. Under both conditions a linear increase in MFD of the dominant follicle was found, preceding as well as following the moment of HCG administration. The follicular growth rate in CC stimulated cycles was increased in the late follicular phase when compared with spontaneous cycles, resulting in significantly larger follicles on days -2, -1 and on day 0, the day of follicle aspiration. This increased growth rate was found under both monofollicular and multifollicular situations. The administration of HCG does not alter the linear increase in MFD as established earlier in the follicular phase in CC stimulated cycles.

Finally, this thesis comprises three studies on the relationship between single plasma E2 values or plasma E2 profiles and pregnancy prospects in CC and gonadotrophins stimulated cycles for IVF purposes. Although it was concluded in a small CC stimulated series, that pregnancy cycles seem to be associated with plasma E2 levels increasing up to the day following HCG administration, in a more extensive study regarding gonadotrophins stimulated cycles such a relationship could not be established. IVF pregnancies occurred in the presence of a wide range of plasma E2 levels during the three days period preceding follicle aspiration. Moreover, the influence of plasma E2 profiles during the late follicular phase on treatment outcome was found to be extremely limited in a large IVF population. Therefore it may be concluded that plasma E2 determinations during IVF cycles monitored by means of ultrasonographical follicle measurements are of little to no importance.

SUMMARY

Chapter 1

Chapter 1 comprises literature on the role of ultrasonography of Graafian follicular growth and of plasma E2 determinations during the follicular phase in the analysis and treatment of infertile women, particularly during IVF treatment cycles. Furthermore, reports on the value of urinary and plasma LH determinations, cervical mucus changes and karyopyknotic index for the evaluation of the natural and stimulated follicular phase are discussed.

The objective of the present study was threefold: (i) To assess accuracy and reproducibility of ultrasonographic follicle measurements performed by both static B-compound and real-time sector scanning techniques; (ii) To define follicular growth rate patterns by means of ultrasonography in stimulated menstrual cycles both preceding and following the administration of HCG and to compare these patterns with those observed in the natural cycle; and (iii): To assess the value of plasma E2 measurements during the follicular phase of stimulated cycles for IVF purposes in relation to treatment outcome.

Chapter 2

Both studies included in Chapter 2 confirm previous and later reports on accuracy and reproducibility of ultrasonographical follicle measurements. Static B-compound and real-time sector scanning techniques provide accurate and reproducible information on the number of follicles, their localization, size and growth rate. As the latter technique is less time consuming, virtually all centres have changed to this kind of ultrasonography. Although in previous reports real-time linear-array systems were used for follicle measurements, now there is general agreement that real-time sector scanners are more appropriate.

Chapter 3

In Chapter 3 two studies on ultrasonographical aspects of follicular growth in CC stimulated cycles are presented. The growth rate values established in the late follicular phase under both monofollicular and multifollicular conditions in the CC stimulated cycle are related to growth rate values in the natural cycle.

The daily increase in MFD of the dominant follicle in the late follicular phase follows a linear pattern in both the natural and CC stimulated cycle up to the estimated moment of ovulation and the moment of follicle aspiration, respectively.

Follicular growth rate in CC stimulated cycles is increased in the late follicular phase when compared with spontaneous cycles, resulting in significantly larger follicles during the late follicular phase. This increased growth rate is found both under monofollicular and multifollicular conditions. Close observation of follicular growth during the 24 h period preceding follicle aspiration in CC stimulated cycles shows a continuous linear increase in MFD, thus allowing prediction of follicular size at follicle aspiration. In agreement with other reports, a wide range of ultimate preovulatory follicle diameters is found.

Chapter 4

Chapter 4 comprises three studies on the value of plasma E2 determinations for prediction of the prospects of a pregnancy in CC or gonadotrophins stimulated cycles for IVF purposes.

Although it is suggested in Section 4.3 that the daily relative increase in plasma E2 values might be the most relevant aspect of plasma E2 monitoring, in Section 4.5 it is concluded from a more extensive series that pregnancy rates are virtually the same, irrespective of the E2 profile following the administration of HCG. Finally it is concluded from 458 treatments cycles, resulting in 102 clinical pregnancies, that pregnancies are equally distributed over a wide range of plasma E2 levels during the three days preceding follicle aspiration.

The influence of plasma E2 determinations on treatment policy therefore must be reconsidered

SAMENVATTING

Hoofdstuk 1

In hoofdstuk 1 zijn literatuurgegevens samengevat met betrekking tot de rol van ultrageluidonderzoek van Graafse follikels en van plasma 17β-oestradiol-bepalingen tijdens de folliculaire fase van de menstruele cyclus van onvruchtbare vrouwen. Vooral gegevens betreffende de behandeling met in-vitro-fertilisatie en embryotransplantatie zijn in de beschouwingen opgenomen. Tevens worden studies besproken over de waarde van LH-bepalingen in urine en plasma, van de cyclische veranderingen van het cervixslijm en van de karyopyknotische index bij het onderzoek van de folliculaire fase, zowel gedurende de spontane cyclus, als tijdens de geïnduceerde menstruele cyclus.

Het doel van het eigen onderzoek was drieledig: (i) het bepalen van de nauwkeurigheid en reproduceerbaarheid van metingen van de Graafse follikel met behulp van B-compound en real-time sector ultrageluidstechniek; (ii) het langs echoscopische weg vaststellen van groeipatronen van de Graafse follikel in de geïnduceerde menstruele cyclus, zowel voorafgaande aan, als volgend op de toediening van HCG, teneinde deze groeipatronen te vergelijken met die waargenomen in de spontane cyclus; (iii) het bepalen van het verband tussen plasma E2-bepalingen tijdens de folliculaire fase van ten behoeve van in-vitro-fertilisatie gestimuleerde cycli en het behandelingsresultaat.

Hoofdstuk 2

Literatuurgegevens over de nauwkeurigheid en reproduceerbaarheid van follikelmetingen met behulp van ultrageluid worden bevestigd door de twee studies in Hoofdstuk 2. Zowel met B-compound als real-time sector scan apparatuur blijkt betrouwbare informatie te worden verkregen over het aantal aanwezige follikels, hun localisatie, grootte en groeisnelheid. Vrijwel alle klinieken geven voor follikelmetingen aan de real-time sector scan techniek de voorkeur, mede omdat deze minder tijdrovend is dan de B-compound scan techniek. Hoewel in oudere studies ook wel real-time linear-array apparatuur werd toegepast, beschouwt men de real-time sector scan techniek bij uitstek geschikt voor follikelmetingen.

Hoofdstuk 3

Hoofdstuk 3 omvat een tweetal studies, waarin de dagelijkse toename in diameter van de dominante follikel in het tweede deel van de folliculaire fase

van de cyclus wordt onderzocht in met clomifeencitraat gestimuleerde cycli, in aanwezigheid van zowel één als meer Graafse follikels. De groeisnelheid wordt vergeleken met die in de spontane cyclus.

Zowel in de spontane als in de met clomifeencitraat gestimuleerde cyclus blijkt de gemiddelde folliculaire diameter in het tweede deel van de folliculaire fase op lineaire wijze toe te nemen tot aan het geschatte ovulatietijdstip, dan wel de follikelaspiratie. De groeisnelheid van de follikel in de met clomifeencitraat gestimuleerde cyclus is groter dan die in de spontane cyclus, hetgeen leidt tot significant grotere dominante follikels tijdens het laatste deel van de folliculaire fase. Deze grotere groeisnelheid wordt zowel in aanwezigheid van één als van meer follikels waargenomen. Ook in de 24 uur voorafgaande aan de follikel-aspiratie en derhalve volgend op de HCG-toediening vertoont de dominante follikel in met clomifeencitraat gestimuleerde cycli een voortzetting van de lineaire toename van de gemiddelde folliculaire diameter, waardoor een voorspelling van de follikelgrootte bij punctie kan worden gedaan. Evenals anderen vonden ook wij een grote spreiding in afmetingen van de preovulatoire follikel.

Hoofdstuk 4

In hoofdstuk 4 worden drie studies beschreven over de relatie tussen plasma 17β-oestradiolbepalingen en de kans op het ontstaan van een zwangerschap in met clomifeencitraat of gonadotrofinen gestimuleerde in-vitro-fertilisatie cycli.

Hoewel het in paragraaf 4.3 lijkt dat de dagelijkse relatieve toename in plasma 17β-oestradiolwaarden het meest belangrijke aspect vormt van het vervolgen van deze steroidbepalingen, blijkt in paragraaf 4.5 in een veel grotere studiegroep dat de zwangerschapskans vrijwel gelijk is, ongeacht de veranderingen in plasma 17β-oestradiolspiegels na de toediening van HCG. Tenslotte blijkt uit 458 in-vitro-fertilisatie behandelingen, die aanleiding hebben gegeven tot 102 klinische zwangerschappen, dat de plasma 17β-oestradiolwaarden in deze 102 cycli in de drie dagen voorafgaande aan de follikelaspiratie in gelijke mate zijn verdeeld over de totale range van plasma 17β-oestradiolspiegels.

Dientengevolge moet de betekenis van plasma 17β-oestradiolbepalingen voor het behandelingsbeleid worden heroverwogen.

REFERENCES

Abdalla, H.I., Ah-Moye, M., Brinsden, P., Howe, D.L., Okonofua, F., Craft, I.: The effect of the dose of human chorionic gonadotropin and the type of gonadotropin stimulation on oocyte recovery rates in an in-vitro fertilization program.
Fertil Steril 48:958, 1987

Abdulwahid, N.A., Adams, J., van der Spuy, Z.M., Jacobs, H.S.: Gonadotrophin control of follicular development.
Clin Endocrin 23:613, 1985

Alberda, A.Th., Leerentveld, R.A., Janssen-Caspers, H.A.B., Scholtes, M.C.W., van Os, H.C., Zeilmaker, G.H.: Resultaten van in-vitro-fertilisatie in het academisch ziekenhuis Rotterdam-Dijkzigt in 1986.
Ned Tijdschr v Geneesk 131:2194, 1987

Bagshawe, K.D., Wilde, C.E., Orr, A.H.: Radioimmunoassay for human chorionic gonadotrophin and luteinizing hormone.
Lancet i:1118, 1966

Baird, D.T., Fraser, I.S.: Blood production and ovarian secretion rates of estradiol and estrone in women throughout the menstrual cycle.
J Clin Endocrin Metab 38:1009, 1974

Bayly, C.M., McBain, J.C., Clarke, G.A., Gronow, M.J., Johnston, W.I.H., Martin, M.J., Speirs, A.L.: Ovarian stimulation regimens in an in-vitro fertilization program: a comparative analysis.
Ann NY Ac Sci 442:123, 1985

Beck, L., Terinde, R.: Diagnostik der Ovulation durch Ultraschall.
Deutsche Med Wochenschr 106:1363, 1981

Ben-Rafael, Z., Kopf, G.S., Blasco, L., Flickinger, G.L., Tureck, R.W., Strauss, J.F., Mastroianni, L.: Follicular maturation parameters, associated with the failure of oocyte retrieval, fertilization, and cleavage in-vitro.
Fertil Steril 45:51, 1986

Bernardus, R.E., Jones, G.S., Acosta A.A., Garcia. J.E., Liu H-C., Jones, D.L., Rosenwaks, Z.: The significance of the ratio in follicle-stimulating hormone and luteinizing hormone in induction of multiple follicular growth.
Fertil Steril 43:373, 1985

Biggs, J.S.G., Cooper, D.H., Miklosi, S., McKenzie, E.: Ultrasound imaging in the management of ovulation stimulation.
Austr N Z J Obstet Gynaecol 24:276, 1984

Black, W.P., Coutts, J.R.T., Dodson, K.S., Rao, L.G.S.: An assessment of urinary and plasma steroid estimations for monitoring treatment of ovulation with gonadotrophins.
J Obstet Gynaecol Brit Cwlth 81:667, 1974

Bordt, J., Hanker, J.P., Schneider, H.P.G.: Ultrasound-controlled gonadotropin therapy of anovulatory infertility.
Fertil Steril 46:818, 1986

Braat, D.D.M., Boghelman, D., Coelingh Bennink, H.J.T., Heineman, M.J., Lappöhn, R.E., Rolland, R., Willemsen, W.N.P., Schoemaker, J.: The outcome of pregnancies established in GnRH induced cycles with special reference to the multiple pregnancies in 5 Dutch centres.
In: Coelingh Bennink, H.J.T., Dogterom, A.A., Lappöhn, R.E., Rolland, R., Schoemaker, J. (Eds), Pulsatile GnRH 1985, proceedings of the 3rd Ferring Symposium. Ferring, Haarlem, The Netherlands: pp 207-215, 1986

Bryce, R.L., Picker, R.H., Saunders, D.M.: Ultrasound in gonadotrophin therapy: a better predictor of ovarian hyperstimulation?
Austr N Z J Obstet Gynaecol 21:237, 1981

Bryce, R.L., Shuter, B., Sinosich, M.J., Stiel, J.N., Picker, R.H., Saunders, D.M.: The value of ultrasound, gonadotropin, and estradiol measurements for precise ovulation prediction.
Fertil Steril 37:42, 1982

Brown, J.B.: Urinary excretion of oestrogens during the menstrual cycle.
Lancet i:320, 1955

Brown, J.B., Beischer, N.A.: Current status of estrogen assay in gynecology and obstetrics.
Obstet Gynecol Surv 27:205, 1972

Buxton, C.L.: The pitfalls of clinical research.
J Clin Endocrinol Metab 13:231, 1953

Buttery, B., Trounson, A., McMaster, R., Wood, C.: Evaluation of diagnostic ultrasound as a parameter of follicular development in an in-vitro fertilization program.
Fertil Steril 39:458, 1983

Cabau, A., Bessis, R.: Monitoring of ovulation induction with human menopausal gonadotropin and human chorionic gonadotropin by ultrasound.
Fertil Steril 36:178, 1981

Carson, R.S., Trounson, A.O., Findlay, J.K.: Successful fertilization of human oocytes in-vitro: concentration of estradiol-17β, progesterone and androstenedione in the antral fluid of donor follicles.
J Clin Endocrinol Metab 55:798, 1982

Cohen, J., Debache, C., Pez, J.P., Junca, A.M., Cohen-Bacrie, P.: Transvaginal sonographically controlled ovarian puncture for oocyte retrieval for in-vitro fertilization.
J in Vitro Fert Embryo Transfer 3:309, 1986

Coulam, C.B., Hill, L.M., Breckle, R.: Ultrasonic evidence for luteinization of unruptured preovulatory follicles.
Fertil Steril 37:524, 1982

de Crespigny, L.Ch., O'Herlihy, C., Robinson, H.P.: Ultrasonic observation of the mechanism of human ovulation.
Am J Obstet Gynecol 139:636, 1981a

de Crespigny, L.J.Ch., O'Herlihy, C., Hoult, I., Robinson, H.P.: Ultrasound in an in-vitro fertilization program.
Fertil Steril 35:25, 1981b

Crowley jr, W.F., Filicori, M., Spratt, D., Santoro, N.: The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women.
Recent Prog Horm Res 41:473, 1985

DeCherney, A.H., Romero, R., Polan, M.L.: Ultrasound in reproductive endocrinology.
Fertil Steril 37:323, 1982

Dellenbach, P., Nisand, I., Moreau, L., Feger, B., Plumere, C., Gerlinger, P., Brun, B., Rumpler, Y.: Transvaginal, sonographically controlled ovarian follicle puncture for egg retrieval.
Lancet i:1467, 1984

Dellenbach, P., Nisand, I., Moreau, L., Feger, B., Plumere, C., Gerlinger, P.: Transvaginal sonographically controlled follicle puncture for oocyte retrieval.
Fertil Steril 44:656, 1985

Diamond, M.P., Webster, B.W., Garner, C.H., Vaughn, W.K., Maxson, W.S., Herbert, C.M., Osteen, K.G., Rogers, B.J., Wentz, A.C.: Selection of superior stimulation protocols for follicular development in a program for in-vitro fertilization.
Fertil Steril 43:251, 1985

Diamond, M.P., Hill, G.A., Herbert, C.M., Rogers, B.J., Osteen, K.G., Maxson, W.S., Wentz, A.C.: Comparison of human menopausal gonadotropin, clomiphene citrate, and combined human menopausal gonadotropin-clomiphene citrate stimulation protocols for in-vitro fertilization.
Fertil Steril 46:1108, 1986

Dirnfeld, M., Lejeune, B., Camus, M., Vekemans, M., Leroy, F.: Growth rate of follicular estrogen secretion in relation to the outcome of in-vitro fertilization and embryo replacement.
Fertil Steril 43:379, 1985

Dlugi, A.M., Laufer, N., DeCherney, A.H., McLusky, N.J., Haseltine, F.P., Polan, M.L., Mezer, H.C., Tarlatzis, B., Naftolin, F.: The periovulatory and luteal phase of conception cycles following in-vitro fertilization and embryo transfer.
Fertil Steril 41:530, 1984

Donald, I.: The use of ultrasonics in the diagnosis of abdominal swellings.
Br Med J 2:1154, 1963

Dor, J., Rudak, E., Mashiach, S., Nebel, L., Serr, D.M., Goldman, B.: Periovulatory 17β-estradiol changes and embryo morphologic features in conception and nonconception cycles after human in-vitro fertilization.
Fertil Steril 45:63, 1986

Edwards, R.G., Steptoe, P.C., Purdy, J.M.: Establishing full-term human pregnancies using embryos grown in-vitro.
Br J Obstet Gynaecol 87:737, 1980

Edwards, R.G.: Test-tube babies, 1981.
Nature 293:253, 1981

Edwards, R.G., Fishel, S.B., Cohen, J., Fehilly, C.B., Purdy, J.M., Slater, J.M., Steptoe, P.C., Webster, J.M.: Factors influencing the success of in-vitro fertilization for alleviating human infertility.
J in Vitro Fert Embryo Transfer 1:3, 1984

Eibschitz, I., Belaisch-Allart, J.C., Frydman, R.: In-vitro fertilization management and results in stimulated cycles with spontaneous luteinizing hormone discharge.
Fertil Steril 45:231, 1986

Eissa, M.K., Hudson, K., Docker, M.F., Sawers, R.S., Newton, J.R.: Ultrasound follicle diameter measurement: an assessment of interobserver and intraobserver variation.
Fertil Steril 44:751, 1985

Engel, T., Jewelewicz, R., Dyrenfurth, I., Speroff, L., vande Wiele, R.L.: Ovarian hyperstimulation syndrome.
Am J Obstet Gynecol 112:1052, 1972

Feichtinger, W., Kemeter, P.: Laparoscopic or ultrasonically guided follicle aspiration for in-vitro fertilization?
J in Vitro Fert Embryo Transfer 1:244, 1984

Feichtinger, W., Kemeter, P.: Transvaginal sector scan sonography for needle guided transvaginal follicle aspiration and other applications in gynecologic routine and research.
Fertil Steril 45:722, 1986

Ferin, M., Rosenblatt, H., Carmel, P.W., Antunes, J.L., VandeWiele, R.L.: Estrogen-induced gonadotropin surges in female rhesus monkeys after pituitary stalk resection.
Endocrinology 104:50, 1979

Ferraretti, A.P., Garcia, J.E., Acosta, A.A., Seegar Jones, G.: Serum luteinizing hormone during ovulation induction with human menopausal gonadotropin for in-vitro fertilization in normally menstruating women.
Fertil Steril 40:742, 1983

Fleischer, A.C., Daniell, J.F., Rodier, J., Lindsay, A.M., James, A.E.: Sonographic monitoring of ovarian follicular development. J Clin Ultrasound 9:275, 1981

Fleischer, A.C., Herbert, C.M., Sacks, G.A., Colston Wentz, A., Entman, S.S., James, A.E.: Sonography of the endometrium during conception and nonconception cycles of in-vitro fertilization and embryo transfer.
Fertil Steril 46:442, 1986

Flynn, A.M., Bertrand, P.V.: The value of a cervical score in the assessment of ovarian function.
J Obstet Gynaecol Br Cwlth 80:152, 1973

Forman, R., Fries, N., Testart, J., Belaisch-Allart, J., Hazout, A., Frydman, R.: Evidence for an adverse effect of elevated serum estradiol concentrations on embryo implantation.
Fertil Steril 49:118, 1988

Freundl, G., Terinde, R., Distler, W., Baumeister, A., Herberger, J., Kozlowski, P.: Serum levels of oestradiol-17β and luteinizing hormone related to follicular growth as monitored by ultrasound in women with normal menstrual cycles.
Acta Endocrin 96:370, 1981

Friedrich, W., Lisse, K., Körner, H., Wilken, Th.: The follicular phase stimulated by human pituitary gonadotrophin and the luteal phase following in-vitro fertilization.
Exp Clin Endocrin 87:1, 1986

Frydman, R., Rainhorn, J-D., Forman, R., Belaisch-Allart, J., Fernandez, H., Lassalle, B., Testart, J.: Programmed oocyte retrieval during routine laparoscopy and embryo cryopreservation for later transfer.
Am J Obstet Gynecol 155:112, 1986a

Frydman, R., Forman, R., Rainhorn, J-D., Belaisch-Allart, J., Hazout, A., Testart, J.: A new approach to follicular stimulation for in-vitro fertilization: programmed oocyte retrieval.
Fertil Steril 46:657, 1986b

Garcia, J.E., Seegar Jones, G., Acosta, A.A., Wright jr., J.: Human menopausal gonadotropin /human chorionic gonadotropin follicular maturation for oocyte aspiration: Phase I, 1981.
Fertil Steril 39:167, 1983a

Garcia, J.E., Seegar Jones, G., Acosta, A.A., Wright jr., J.: Human menopausal gonadotropin /human chorionic gonadotropin follicular maturation for oocyte aspiration: Phase II, 1981.
Fertil Steril 39:174, 1983b

Gemzel, C.A., Diczfalusy, E., Tillinger, K.G.: Clinical effect of human pituitary follicle stimulating hormone.
J Clin Endocrinol Metab 18:1333, 1958

Geisthövel, F., Skubsch, U., Zabel, G., Schillinger, H., Breckwoldt, M.: Ultrasonographic and hormonal studies in physiologic and insufficient menstrual cycles.
Fertil Steril 39:277, 1983

Gidley-Baird, A.A., O'Neill, C., Sinosich, M.J., Porter, R.N., Pike, I.L., Saunders, D.M.: Failure of implantation in human in-vitro fertilization and embryo transfer patients: the effects of altered progesterone /estrogen ratios in humans and mice.
Fertil Steril 45:69, 1986

Glasier, A., Thatcher, S.S., Wickings, E.J., Hillier, S.G., Baird, D.T.: Superovulation with exogenous gonadotropins does not inhibit the luteinizing hormone surge.
Fertil Steril 49:81, 1988

Gleicher, N., Friberg, J., Fullan, N., Giglia, R.V., Mayden, K., Kesky, T., Siegel, I.: Egg retrieval for in-vitro fertilization by sonographically controlled vaginal culdocentesis.
Lancet ii:508, 1983

Glissant, A., de Mouzon, J., Frydman, R.: Ultrasound study of the endometrium during in-vitro fertilization cycles.
Fertil Steril 44:786, 1985

Greenblatt, R.B., Barfiel, W.E., Jungck, E.C., Ray, A.W.: Induction of ovulation with MRL-41.
JAMA 178:101, 1961

Hackelöer, B.J., Nitschke, S., Daume, E., Buchholz, R.: Ultraschalldarstellung von Ovarveränderungen bei Gonadotropinstiemulierung.
Geburtsh u Frauenheilk 37:185, 1977

Hackelöer, B.J., Robinson, H.P.: Ultraschalldarstellung des wachsenden Follikels und Corpus Luteum im normalen physiologischen Zyklus.
Geburtsh u Frauenheilk 38:163, 1978

Hackelöer, B.J.: Ultrasonic demonstration of follicular development.
Lancet i:941, 1978

Hackelöer, B.J., Fleming, R., Robinson, H.P., Adam, A.H., Coutts, J.R.T.: Correlation of ultrasonic and endocrinologic assessment of human follicular development.
Am J Obstet Gynecol 135:122, 1979

Hackelöer, B.J., Nitschke-Dabelstein, S.: Ultrasonic monitoring of ovarian structural changes.
In: Kurjak, A. (Ed), Progress in medical ultrasound. Amsterdam: Excerpta Medica, pp 141-154, 1980

Hackelöer, B.J.:
Personal communication, 1982

Hackelöer, B.J.: Ultrasound scanning of the ovarian cycle.
J in Vitro Fert Embryo Transfer 1:217, 1984a

Hackelöer, B.J.: The role of ultrasound in female infertility management.
Ultrasound Med Biol 10:35, 1984b

Hall, D.A., Hann, L.E., Ferrucci J.T., Black, E.B., Braitman, B.S., Crowley, W.F., Nikrui, N., Kelley, J.A.: Sonographic morphology of the normal menstrual cycle.
Radiology 133:185, 1979

Hamblen, E.C., Davis, C.D.: Treatment of hypoovarianism by the sequential and cyclic administration of equine and chorionic gonadotropins.
Am J Obstet Gynecol 50:137, 1945

Hamilton, C.J.C.M., Wetzels, L.C.G., Evers, J.L.H., Hoogland, H.J., Muijtjens, A., de Haan, J.: Follicle growth curves and hormonal patterns in patients with the luteinized unruptured follicle syndrome.
Fertil Steril 43:541, 1985

Hamilton, C.J.C.M., Evers, J.L.H., de Haan, J.: Ultrasound increases the prognostic value of the postcoital test.
Gynecol Obstet Invest 21:80, 1986

Hamilton, C.J.C.M., Evers, J.L.H., Tan, F.E.S., Hoogland, H.J.: The reliability of ovulation prediction by a single ultrasonographic follicle measurement.
Hum Reprod 2:103, 1987a

Hamilton, C.J.C.M., Evers, J.L.H., de Haan, J.: Ovulatory disturbances in patients with luteal insufficiency.
Clin Endocrinol 26:129, 1987b

Haning, R.V., Austin, C.W., Kuzma, D.L., Shapiro, S.S., Zweibel, W.J.: Ultrasound evaluation of estrogen monitoring for induction of ovulation with menotropins.
Fertil Steril 37:627, 1982

Haning, R.V., Austin, C.W., Carlson, I.H., Kuzma, D.L., Shapiro, S.S., Zweibel, W.J.: Plasma estradiol is superior to ultrasound and urinary estriol glucuronide as a predictor of ovarian hyperstimulation during induction of ovulation with menotropins.
Fertil Steril 40:31, 1983

Hill, G.A., Diamond, M.P., Maxson, W.S., Herbert, C.M., Webster, B.W., Vaughn, W.K., Osteen, K.G., Rogers, B.J., Wentz, A.C.: Combination clomiphene citrate /human menopausal gonadotropin stimulation for in-vitro fertilization-embryo transfer.
J in Vitro Fert Embryo Transfer 4:34, 1987

Hill, L.M., Breckle, R., Coulam, C.B.: Assessment of human follicular development by ultrasound.
Mayo Clin Proc 57:176, 1982

Hoff, J.D., Quigley, M.E., Yen, S.S.C.: Hormonal dynamics at midcycle: a reevaluation.
J Clin Endocr Metab 57:792, 1983

Honda, I., Uchimura, M., Kobayashi, Y., Inoue, M., Fujii, A.: Monitoring of the preovulatory follicles - a comparison of ultrasound and laparoscopy.
Acta Obstet Gynaecol Jpn 36:421, 1984

Honda, I., Matsuura, S., Kobayashi, Y., Inoue, M., Fujii, A.: Collection of human oocytes by percutaneous aspiration using ultrasound.
Acta Obstet Gynaecol Jpn 37:1007, 1985

Hoult, I., de Crespigny, L.Ch., O'Herlihy, C., Speirs, A.L., Lopata, A., Kellow, G., Johnston, I., Robinson, H.P.: Ultrasound control of clomiphene /human chorionic gonadotropin stimulated cycles for oocyte recovery and in-vitro fertilization.
Fertil Steril 36:316, 1981

Howles, C.M., Macnamee, M.C., Edwards, R.G.: Follicular development and early luteal function of conception and non-conception cycles after human in-vitro fertilization: endocrine correlates.
Hum Reprod 2:17, 1987

Hühner, M.: Sterility in the male and the female and its treatment.
New York: Rebman, 1913

Hull, M.G.R.: Ovulation failure and induction.
Clinics Obstet Gynaecol 8:753, 1981

Hull, M.E., Moghissi, K.S., Magyar, D.M., Hayes, M.F., Zador, I., Olson, J.M.: Correlation of serum estradiol levels and ultrasound monitoring to assess follicular maturation.
Fertil Steril 46:42, 1986

Hünlich, T., Trotnow, S., Mulz, D., Kniewald, T.: Hormonal and ultrasonic characteristics of 23 clomiphene citrate stimulated cycles resulting in pregnancies after in-vitro fertilization and embryo transfer.
Arch Gynecol 236:19, 1984

Insler, V., Melmed, H., Eichenbrenner, I., Serr, D.M., Lunenfeld, B.: A simple semiquantitative method for monitoring of the menstrual cycle.
Int J Gynaecol Obstet 10:223, 1972

Jacobs, H.S.: Induction of ovulation in women using pulsatile LHRH-clinical results.
In: Bloom, S.R., Jacobs, H.S. (Eds), Therapeutic applications of LHRH. London: Royal Society of Medicine Services, pp 99-109, 1986

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

Janssen-Caspers, H.A.B., Kruitwagen, R.F.P.M., Wladimiroff, J.W., de Jong, F.H., Drogendijk, A.C.: Diagnosis of luteinized unruptured follicle syndrome by ultrasound and steroid hormone assays in peritoneal fluid: a comparative study.
Fertil Steril 46:823, 1986a

Janssen-Caspers, H.A.B., van Gent, I., Wladimiroff, J.W., Alberda, A.Th., Zeilmaker, G.H.: Ultrasonically guided percutaneous and laparoscopic oocyte aspiration: a comparative study.
Eur J Obstet Gynecol Reprod Biol 21:219, 1986b

Janssen-Caspers, H.A.B., Wladimiroff, J.W., van Gent, I., Alberda, A.Th., Leerentveld, R.A., Zeilmaker, G.H., Drogendijk, A.C.: Ultrasonically guided percutaneous and transvaginal aspiration: a comparative study.
Human Reprod 3:337, 1988

Jewelewicz, R.: Management of infertility resulting from anovulation.
Am J Obstet Gynecol 122:909, 1975

Johnson, M., Graham, M., Cooperburg, P.: Abnormal endometrial echoes: Sonographic spectrum of endometrial pathology.
J Ultrasound Med 1:161, 1982

Jones jr, H.W., Seegar Jones G., Andrews M.C., Acosta A., Bundrun, C., Garcia, J., Sandow, B., Veeck, L., Wilkes, C., Witmyer, J., Wortham, J.E., Wright, G.: The program for in-vitro fertilization at Norfolk.
Fertil Steril 38:14, 1982

Jones jr., H.W., Acosta, A., Andrews, M.C., Garcia, J.E., Seegar Jones, G., Mantzavinos, T., McDowell, J., Sandow, B., Veeck, L., Whibley, T., Wilkes, C., Wright, G.: The importance of the follicular phase to success and failure in in-vitro fertilization.
Fertil Steril 40:317, 1983

Jones jr., H.W., Acosta, A.A., Andrews, M.C., Garcia, J.E., Seegar Jones, G., Mayer, J., McDowell, J.S., Rosenwaks, Z., Sandow, B.A., Veeck, L.L., Wilkes, C.A.: Three years of in-vitro fertilization at Norfolk.
Fertil Steril 42:826, 1984

Kemeter, P., Feichtinger, W.: Transvaginal oocyte retrieval using a transvaginal sector scan probe combined with an automated puncture device.
Hum Reprod 1:21, 1986

Kerin, J.F.: Determination of the optimal timing of insemination in women.
In: Richardson, D., Joyce, D., Symonds, M. (Eds), Frozen human semen. London, RCOG, pp 105-132, 1979

Kerin, J.F., Warnes, G.M., Crocker, J., Broom, T.G., Ralph, M.M., Matthews, C.D., Seamark, R.F., Cox, L.W.: 3-hour urinary radio immunoassay for luteinizing hormone to detect onset of preovulatory LH surge.
Lancet ii:430, 1980

Kerin, J.F., Edmonds D.K., Warnes, G.M., Cox, L.W., Seamark, R.F., Matthews, C.D., Young, G.B., Baird, D.T.: Morphological and functional relations of graafian follicle growth to ovulation in women using ultrasonic, laparoscopic and biochemical measurements.
Br J Obstet Gynaecol 88:81, 1981

Kerin, J.F., Kirby, C., Morris, D., McEvoy, M., Ward, B., Cox, L.W.: Incidence of the luteinized unruptured follicle phenomenon in cycling women.
Fertil Steril 40:620, 1983

Killick, S., Elstein, M.: Pharmacologic production of luteinized unruptured follicles by prostaglandin synthetase inhibitors.
Fertil Steril 47:773, 1987

Knobil, E.: The neuroendocrine control of the menstrual cycle.
Rec Prog Horm Res 36:53, 1980

Koninckx, P.R., Heyns, W.J., Corvelyn, P.A., Brosens, I.A.: Delayed onset of luteinization as a cause of infertility.
Fertil Steril 29:266, 1978

Kratochwil, A., Urban, G., Friedrich, F.: Ultrasonic tomography of the ovaries.
Ann Chir Gynaecol Fenn 61:211, 1972

Kratochwil, A., Jentsch, K., Bresina, K.: Ultraschall-anatomie des weiblichen Beckens und ihre klinische Bedeutung.
Arch Gynaekol 214:273, 1973

Laufer, N., Haseltine, F.P., DeCherney, A.H.: The value of serum 17β-estradiol in predicting follicular maturation in cycles induced by hMG /hCG treatment is limited.
Fertil Steril (Abstr) 39:415, 1983a

Laufer, N., DeCherney, A.H., Haseltine, F.P., Polan, M.L., Mezer, H.C., Dlugi, A.M., Sweeney, D., Nero, F., Naftolin, F.: The use of high-dose human menopausal gonadotropin in an in-vitro fertilization program.
Fertil Steril 40:734, 1983b

Laufer, N., DeCherney A.H., Tarlatzis B.C., Naftolin, F.: The association between preovulatery 17β-estradiol pattern and conception in human menopausal gonadotropin-human chorionic gonadotropin stimulation.
Fertil Steril 46:73, 1986

Leerentveld, R.A., Van Gent, I., Alberda, A.Th., Wladimiroff, J.W.: Assessment of follicular development in clomiphene induced cycles by means of ultrasound and laparoscopy: a comparative study.
Ultrasound Med Biol 9:595, 1983

Leerentveld, R.A., van Gent, I., van der Stoep, M., Alberda, A.Th.: Comparison of Graafian follicle dimensions as determined by static and real-time sector scanning.
Fertil Steril 42:929, 1984

Leerentveld, R.A., van Gent, I., van der Stoep, M., Wladimiroff, J.W.: Ultrasonographic assessment of graafian follicle growth under monofollicular and multifollicular conditions in clomiphene citrate-stimulated cycles.
Fertil Steril 43:565, 1985

Leerentveld, R.A., Wladimiroff, J.W.: 24-h echographic profile study on follicular growth prior to laparoscopic aspiration of the graafian follicle in clomiphene citrate /human chorionic gonadotrophin-stimulated cycles.
Eur J Obstet Gynecol Reprod Biol 22:287, 1986

Leerentveld, R.A., Zeilmaker, G.H., van der Stoep, M., Wladimiroff, J.W., de Jong, F.H., Alberda, A.Th.: Monitoring of clomiphene stimulation by means of ultrasonographic follicle measurements in in-vitro fertilization treatment cycles.
Hum Reprod 2:187, 1987

Leerentveld, R.A., Janssen-Caspers, H.A.B., van Os, H.C., Scholtes, M.C.W., Wladimiroff, J.W., Zeilmaker, G.H., de Jong, F.H., Alberda, A.Th.: The value and role of plasma 17β-oestradiol measurements during ovarian hyperstimulation for in-vitro fertilization.
Accepted for publication in Human Reproduction, 1988

Lemay, A., Bastide, R., Lambert, R., Rioux, J.E.: Prediction of human ovulation by rapid luteinizing hormone (LH) radioimmunoassay and ovarian ultrasonography.
Fertil Steril 38:194, 1982

Lenz, S., Lauritsen J.G., Kjellow, M.: Collection of human oocytes for in-vitro fertilization by ultrasonically guided follicular puncture.
Lancet i:1163, 1981

Lenz, S., Lauritsen, J.G.: Ultrasonically guided percutaneous aspiration of human follicles under local anesthesia: a new method of collecting oocytes for in-vitro fertilization.
Fertil Steril 38:673, 1982

Lenz, S.: Ultrasonically guided aspiration of human oocytes.
Ultrasound Med Biol 10:625, 1984

Lenz, S., Leeton, J., Trounson, A.: Transfundal transfer of embryo's using ultrasound.
J in Vitro Fert Embryo Transfer 4:13, 1987a

Lenz, S., Leeton, J., Renou, P.: Transvaginal recovery of oocytes for in-vitro fertilization using vaginal ultrasound.
J in Vitro Fert Embryo Transfer 4:51, 1987b

Levran, D., Lopata, A., Nayadu, P.L., Martin, M.J., McBain, J.C., Bayly, C.M., Speirs, A.L., Johnston, W.I.H.: Analysis of the outcome of in-vitro fertilization in relation to the timing of human gonadotropin administration by the duration of estradiol rise in stimulated cycles.
Fertil Steril 44:335, 1985

Lewin, A., Laufer, N., Rabinowitz, R., Schenker, J.: Utrasonically guided oocyte recovery for in-vitro fertilization: an improved method.
J in Vitro Fert Embryo Transfer 3:370, 1986a

Lewin, A., Laufer, N., Rabinowitz, R., Margalioth E.J., Bar, I., Schenker, J.: Ultrasonically guided oocyte collection under local anesthesia: the first choice method for in-vitro fertilization-a comparitive study with laparoscopy.
Fertil Steril 46:257, 1986b

Leyendecker, G., Wildt, L.: Die pulsatile Therapie mit Gonadotropin-Releasing-Hormon (GnRH). Geburtsh u Frauenheilk 42:689, 1982

Lopata, A., Johnston, W.I.H., Hoult, I.J., Speirs, A.I.: Pregnancy following intrauterine implantation of an embryo obtained by in-vitro fertilization of a preovulatory egg. Fertil Steril 33:117, 1980

Lopata, A.: Concepts in human in-vitro fertilization and embryo transfer. Fertil Steril 40:289, 1983

Liukkonen, S., Koskimies, A.I., Tenhunen, A., Ylöstalo, P.: Diagnosis of luteinized unruptured follicle (LUF) syndrome by ultrasound. Fertil Steril 41:26, 1984

Lunenfeld, B., Menzi, A., Volet, B.: Clinical effects of a human postmenopausal gonadotropin. Rass Clin Ter Sci Affini 59:213, 1960

Macler, J., Jacquetin, B., Ehret, C., Dervain, I., Plas-Roger, S., Aron, C., Reneaud, R.: La surveillance echographique de l'induction de l'ovulation. J Gynaecol Obstet Biol Reprod 7:746, 1978

Mantzavinos, T., Garcia, J.E., Jones, H.W.: Ultrasound measurement of ovarian follicles stimulated by human gonadotropins for oocyte recovery and in-vitro fertilization. Fertil Steril 40:461, 1983

Marik, J., Hulka, J.: Luteinized unruptured follicle syndrome: a subtle cause of infertility. Fertil Steril 29:270, 1978

Marinho, A.O., Sallam, H.N., Goessens, L., Collins, W.P., Rodeck, C.H., Campbell, S.: Real-time pelvic ultrasonography during the periovulatory period of patients attending an artificial insemination clinic. Fertil Steril 37:633, 1982

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

Mastroianni, L: The role and value, present and future, of ultrasound in the detection of ovulation. Fertil Steril 34:177, 1980

Matson, P.L., Tuvik, A.I., O'Halloran, F., Yovich, J.L.: The value of the postcoital test in predicting the fertilization of human oocytes. J in Vitro Fert Embryo Transfer 3:110, 1986

McArdle, C.R., Seibel, M.M., Weinstein, F., Hann, L.E., Nickerson, C., Taymor M.L.: Induction of ovulation monitored by ultrasound. Radiology 148:809, 1983

McBain, J.C., Trounson, A.O.: Patient management-treatment cycle. In: Wood, C., Trounson, A. (Eds), Clinical in-vitro fertilization. Springer-Verlag, Berlin: pp 49-65, 1984

McBain, J.C., Levran. D., Martin, M.J., Bayly, C.M., Lopata, A., Clarke, G.A., Speirs, A.L., Nayudu, P., Du Plessis, Y.P., Johnston, W.I.H.: An analysis of endocrine indices that may identify conceptual cycles prospectively in an in-vitro fertilization program. Ann N Y Acad Sci 442:140, 1985

McIntosh, J.E.A., Matthews, C.D., Crocker, J.M., Broom, T.J., Cox, L.W.: Predicting the luteinizing hormone surge: relationship between the duration of the follicular and luteal phases and the length of the human menstrual cycle. Fertil Steril 34:125, 1980

McNatty, K.P., Baird, D.T., Bolton, A., Chambers, P., Corker, C.S., McLean, H.: Concentrations of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. J Endocrinol 71:77, 1976

Messinis, I., Templeton, A., Baird, D.T.: Luteal phase after ovarian hyperstimulation. Br J Obstet Gynaecol 94:345, 1987

Mettler. L., Seki, M., Baukloh, V., Semm, K.: Human ovum recovery via operative laparoscopy and in-vitro fertilization.
Fertil Steril 38:30, 1982

Muasher, S.J., Garcia, J.E., Rosenwaks, Z.: The combination of follicle-stimulating hormone and human menopausal gonadotropin for the induction of multiple follicular maturation for in-vitro fertilization.
Fertil Steril 44:62, 1985

Nilsson, L., Wikland, M., Hamberger, L., Hillensjö, T., Chari, S., Sturm, G., Daume, E.: Simplification of the method of in-vitro fertilization: sonographic measurement of follicular diameter as the sole index of follicular maturity.
J in Vitro Fertil Embryo Transfer 2:17, 1985

Nitschke-Dabelstein, S., Sturm, G., Hackelöer, B.J., Daume, E., Buchholz, R.: Welchen Stellenwert besitzt die endokrinologische Überwachung in der Gonadotropin-stiemulierung anovulatorischer Patientinnen-ein Vergleich zwischen endokrinologischen und ultrasonographischen Parametern.
Geburtsh u Frauenheilk 40:702, 1980a

Nitschke-Dabelstein, S., Hackelöer, B.J., Sturm, G.: Ultrasonic monitoring of ovarian stimulating therapy.
In: Kurjak, A. (Ed), Progress in medical ultrasound. Amsterdam: Excerpta Medica, pp 155-168, 1980b

Norman, R.L., Resko, J.A., Spies, H.G.: The anterior hypothalamus: how it affects gonadotropin secretion in the rhesus monkey.
Endocrinology 99:59, 1976

Notation, A.D., Tagatz, G.E., Steffes, M.W.: Serum 17β-estradiol, index of follicular maturation during gonadotrophin therapy.
Obstet Gynecol 51:204, 1978

Odell, W.D., Ross, G.T., Rayford, P.L.: Radioimmunoassay for human luteinizing hormone.
Metab Clin Exptl 15:287, 1966

Oelsner, G., Pan, S.B., Barnea, E.R., Boyers, S.P., Tarlatzis, B.C., DeCherney, A.H.: The value of the cervical score in monitoring ovulation induction for in-vitro fertilization: a prospective double-blind study.
J in Vitro Fert Embryo Transfer 3:366, 1986

O'Herlihy, C., de Crespigny, L.J.Ch., Robinson, H.P.: Monitoring ovarian follicular development with real-time ultrasound.
Br J Obstet Gynaecol 87:613, 1980a

O'Herlihy, C., de Crespigny, L.Ch., Lopata, A., Johnston, I., Hoult, I., Robinson, H.: Preovulatory follicular size: a comparison of ultrasound and laparoscopic measurements.
Fertil Steril 34:24, 1980b

O'Herlihy, C., Pepperell, R.J., Robinson, H.P.: Ultrasound timing of human chorionic gonadotropin administration in clomiphene-stimulated cycles.
Obstet Gynecol 59:40, 1982a

O'Herlihy, C., Evans, J.H., Brown, J.B., de Crespigny L.J.Ch., Robinson, H.P.: Use of ultrasound in monitoring ovulation induction with human pituitary gonadotropins.
Obstet Gynecol 60:577, 1982b

Okamoto, S., Healy, D.L., Howlett, D.T., Rogers, P.A.W., Leeton, J.F., Trounson, A.O., Wood, E.C.: An analysis of plasma estradiol concentrations during clomiphene citrate-human menopausal gonadotropin stimulation in an in-vitro fertilization-embryo transfer program.
J Clin Endocrin Metab 63:736, 1986

Parsons, J., Booker, M., Akkermans, J., Riddle, A., Sharma, V., Wilson, V., Whitehead, M., Campbell, S.: Oocyte retrieval for in-vitro fertilization by ultrasonically guided needle aspiration via the urethra.
Lancet ii:1076, 1985

Pepperell, R.J., Brown, J.B., Evans, J.H., Rennie, G.C., Burger, H.G.: The investigation of ovarian function by measurement of urinary oestrogen and pregnanediol excretion.
Br J Obstet Gynaecol 82:321, 1975

Picker, R.H., Smith, D.H., Tucker, M.H., Saunders, D.M.: Ultrasonic signs of imminent ovulation.
J Clin Ultrasound 11:1, 1983

Polan, M.L., Totora, M., Caldwell, B.V., DeCherney, A.H., Haseltine, F.P., Kase, N.: Abnormal ovarian cycles as diagnosed by ultrasound and serum estradiol levels.
Fertil Steril 37:342, 1982

Prins, G.S., Vogelzang, R.L.: Inherent sources of ultrasound variability in relation to follicular measurements.
J in Vitro Fert Embryo Transfer 1:221, 1984

Queenan, J.T., O'Brien, G.D., Bains, L.M., Simpson, J., Collins, W.P., Campbell, S.: Ultrasound scanning of ovaries to detect ovulation in women.
Fertil Steril 34:99, 1980

Quigley, M.M., Sokoloski, J.E., Richards, S.I.: Timing human chorionic gonadotropin administration by days of estradiol rise.
Fertil Steril 44:791, 1985

Rabinowitz, R., Laufer, N., Lewin, A., Navot, D., Bar, I., Margalioth, E.J., Schenker, J.J.G.: The value of ultrasonographic endometrial measurement in the prediction of pregnancy following in-vitro fertilization.
Fertil Steril 45:824, 1986

Rainhorn, J.-D., Forman, R., Belaisch-Allart, J., Hazout, A., Fries, N., Testart, J., Frydman, R.: One year's experience with programmed oocyte retrieval for IVF.
Hum Reprod 2:491, 1987

Renaud, R., Dervain, I., Macler, J., Ehret, C., Spira, A.: Ultrasound monitoring of ovulation.
Lancet i:665, 1979

Renaud, R.L., Macler, J., Ehret, M.C., Aron, C., Plas-Roser, S., Spira, A., Pollack, H.: Echographic study of follicular maturation and ovulation during the normal menstrual cycle.
Fertil Steril 33:272, 1980

Roberts, A.B., Graham, F.M., Binkerd, P.: Ultrasonic preovulatory follicular appearances as an indicator of the outcome of an in-vitro fertilization cycle.
Fertil Steril 43:560, 1985

Robertson R.D., Picker, R.H., Wilson, P.C., Saunders, D.M.: Assessment of ovulation by ultrasound and plasma estradiol determinations.
Obstet Gynecol 54:686, 1979

Rönnberg, L., Ylöstalo, P., Joupilla, P.: Ultrasound to time insemination.
Lancet i:669, 1978

Ross, G.T., Cargille, C.M., Lipsett, M.B., Rayford, P.L., Marshall, J.R., Strott, C.A., Rodbard, D.: Pituitary and gonadal hormones in women during spontaneous and induced cycles.
Rec Progr Horm Res 26:1, 1970

Russell, J.B., Polan, M.L., DeCherney, A.H.: The use of pure follicle-stimulating hormone for ovulation induction in normal ovulatory women in an in-vitro fertilization program.
Fertil Steril 45:829, 1986

Saaranen, M., Suhonen, M., Saarikoski, S.: Ultrasound in the timing of artificial insemination with frozen donor semen.
Gynecol Obstet Invest 22:140, 1986

Sakamoto, C., Nakano, H.: The echogenic endometrium and alterations during menstrual cycle.
Int J Gynaecol Obstet 20:255, 1982

Sallam, H.N., Marinho, A.O., Collins, W.P., Rodeck, C.H., Campbell, S.: Monitoring gonadotrophin therapy by real-time ultrasonic scanning of ovarian follicles.
Br J Obstet Gynaecol 89:155, 1982

Santoro, N., Filicori, M., Crowley jr, W.F.: Hypoganadotropic disorders in men and women: diagnosis and therapy with pulsatile gonadotropin-releasing hormone.
Endocr Rev 7:11, 1986

Sanyal, M.K., Berger, M.J., Thompson I.E., Taymor, M.L., Horne, H.W.: Development of graafian follicles in adult human ovary. I. Correlation of estrogen and progesterone concentration in antral

fluid with growth of follicles.
J Clin Endocrinol Metab 38:828, 1974

Scheer, K., Goldstein, D.P.: Use of ultrasonography to follow regression of theca lutein cysts.
Radiology 108:673, 1973

Schmidt, W., von Holst, T., Garoff, L., Gabelmann, J., Kubli, F.: Monitoring of hMG-stimulated follicular development by real-time ultrasound.
Eur J Obstet Gynec Reprod Biol 12:95, 1981

Seibel, M.M., McArdle, C.R., Thompson, I.E., Berger, M.J., Taymor, M.L.: The role of ultrasound in ovulation induction: a critical appraisal.
Fertil Steril 36:573, 1981

Serafini, P., Stone, B., Kerin, J., Batzofin, J., Quinn, P., Marrs, R.: Occurrence of a spontaneous luteinizing hormone surge is superovulated cycles-predictive value of serum progesterone.
Fertil Steril 49:86,1988

Shaaban, M.M., Klopper, A.: Plasma oestradiol and progesterone concentration in the normal menstrual cycle.
Br J Obstet Gynaecol 80:776, 1973

Sims, M: Illustrations of the value of the microscope in the treatment of the sterile condition.
Br Med J ii:465, 1868

Smith, D.H., Picker, R.H., Sinosich, M., Saunders, D.M.: Assessment of ovulation by ultrasound and estradiol levels during spontaneous and induced cycles.
Fertil Steril 33:387, 1980

Smith, D.H., Picker, R.H., Sinosich, M.J., Saunders, D.M., Grudzinskas, J.G.: Effects of clomiphene administration on ovarian function as measured by estradiol and ultrasound.
Obstet Gynecol 61:695, 1983

Smith, B., Porter, R., Ahuja, K., Craft, I.: Ultrasonic assessment of endometrial changes in stimulated cycles in an in-vitro fertilization and embryo transfer program.
J in Vitr Fert Embryo Transfer 1:233, 1984

Soules, M.R., Sutton, G.P., Hammond, C.B., Haney, A.F.: Endocrine changes at operation under general anesthesia: reproductive hormone fluctuations in young women.
Fertil Steril 33:364, 1980

Stanger, J.D., Yovich, J.L.: Reduced in-vitro fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase.
Br J Obstet Gynaecol 92:385, 1985

Steptoe, P.C., Edwards, R.G.: Birth after the reimplantation of a human embryo.
Lancet ii:366, 1978

Strickler, R.C., Christianson, C., Crane, J.P., Curato, A., Knight, A.D., Yang, V.: Ultrasound guidance for human embryo transfer.
Fertil Steril 43:54, 1985

Talbert L.M.: Endogenous luteinizing hormone surge and superovulation.
Fertil Steril 49:24, 1988

Tarlatzis, B.C., Laufer, N., DeCherney, A.H.: The use of ovarian ultrasonography in monitoring ovulation induction.
J in Vitro Fert Embryo Transfer 1:226, 1984

Testart, J., Frydman, R., Feinstein, M.C., Thebault, A., Roger, M., Scholler, R.: Interpretation of plasma luteinizing hormone assay for the collection of mature oocytes from women: definition of a luteinizing hormone surge-initiating rise.
Fertil Steril 36:50, 1981

Testart, J., Frydman, R.: Minimum time lapse between luteinizing hormone surge or human gonadotropin administration and follicular rupture.
Fertil Steril 37:50, 1982

Testart, J., Belaisch-Allart, J., Hazout, A., Guillet-Rosso, F., Frydman, R.: Actual methods and results in an IVF and ET program.
In: Rolland, R., Heineman, M.J., Hillier, S.G., Vemer, H. (eds), Gamete quality and fertility regulation. Elsevier, Amsterdam: pp 271-282, 1985

Tredway, D.R., Goebelsmann, U., Thorneycroft, I.H., Mishell, D.R.: Monitoring induction of ovulation with human menopausal gonadotropin by a rapid estrogen radioimmunoassay.
Am J Obstet Gynecol 120:1035, 1974

Trotnow, S., Habermann, P.G., Hünlich, T., Siebzehnrübl, E.: A new set of instruments for sonographically controlled follicular puncture.
Arch Gynecol 236:211, 1985

Trounson, A.O., Leeton, J.F., Wood, C., Webb, J., Wood, J.: Pregnancies in humans by fertilization in-vitro and embryo transfer in the controlled ovulatory cycle.
Science 212:681, 1981

Trounson A.O.: Manipulation of endocrine requirements for in-vitro fertilization.
In: Proceedings of the Australian Society of Reproductive Biology, Suppl. 1: pp 1-6, 1982

Vargyas, J.M., Marrs, R.P., Kletzky, O.A., Mishell, D.R.: Correlation of ultrasonic measurement of ovarian follicle size and serum estradiol levels in ovulatory patients following clomiphene citrate for in-vitro fertilization.
Am J Obstet Gynecol 144:569, 1982

Vargyas, J.M., Morente, C., Shangold, G., Marrs, R.P.: The effect of different methods of ovarian stimulation for human in-vitro fertilization and embryo replacement.
Fertil Steril 42:745, 1984

Vermesh, M., Kletzky, O.A., Davajan, V., Israel, R.: Monitoring techniques to predict and detect ovulation.
Fertil Steril 47:259, 1987

Wardle, P.G., Foster, P.A., Mitchell, J.D., McLaughlin, E.A., Williams, J.A.C., Corrigan, E., Ray, B.D., McDermott, A., Hull, M.G.R.: Norethisterone treatment to control timing of the IVF cycle.
Hum Reprod 1:455, 1986

Wetzels, L.C.G., Hoogland, H.J.: Standaardisatie van echoscopische meting van ovarium follikels.
Ned T v Geneesk 126:739, 1982

Wetzels, L.C.G.: Ultrasonographical aspects of follicle growth.
Thesis, University of Limburg, Maastricht, 1983

WHO, task force on methods for the determination of the fertile period: Temporal relationships between ovulation and defined changes in the concentration of plasma estradiol-17β, luteinizing hormone, follicle stimulating hormone and progesterone. I. Probit analysis.
Am J Obstet Gynecol 138:383, 1980

Wikland, M., Nilsson, L., Hansson, R., Hamberger, L., Janson, P.O.: Collection of human oocytes by the use of sonography.
Fertil Steril 39:603, 1983

Wikland, M., Enk, L., Hamberger, L.: Transvesical and transvaginal approaches for the aspiration of follicles by use of ultrasound.
Ann N Y Acad Sci 442:182, 1985

Wikland, M., Enk, L., Hammarberg, K., Nilsson, L.: Use of a vaginal transducer for oocyte retrieval in an IVF/ET program.
J Clin Ultrasound 15:245, 1987

Wu, C.H.: Plasma hormones in clomiphene citrate therapy.
Obstet Gynecol 49:443, 1977

Yee, B., Barnes, R.B., Vargyas, J.M., Marrs, R.P.: Correlation of transabdominal and transvaginal ultrasound measurements of follicle size and number with laparoscopic findings for in-vitro fertilization.
Fertil Steril 47:828, 1987

Ylöstalo, P., Rönnberg, L., Jouppila, P.: Measurements of the ovarian follicle by ultrasound in ovulation induction.
Fertil Steril 31:651, 1979

Ylöstalo, P., Lindgren, P.G., Nillius, S.J.: Ultrasonic measurement of ovarian follicles, ovarian and uterine size during induction of ovulation with human gonadotrophins.
Acta Endocrin 98:592, 1981

Zarutskie, P.W., Kuzan, F.B., Dixon, L., Soules, M.R.: Endocrine changes in the late-follicular and postovulatory intervals as determinants of the in-vitro fertilization pregnancy rate. Fertil Steril 47:137, 1987

Zeilmaker, G.H.: The laboratory procedure of in-vitro fertilization; some observations and conclusions. In: Hormones and cell regulation. Ed. J. Nunez et al. Colloque INSERM /John Libbey Eurotext Ltd. 139:43, 1986

Zemlyn, S.: Comparison of pelvic ultrasonography and pneumography for ovarian size. J Clin Ultrasound 2:331, 1975

ACKNOWLEDGEMENTS

The study described in this thesis was carried out in the Department of Obstetrics and Gynaecology of the Academic Hospital Rotterdam-Dijkzigt, Rotterdam, The Netherlands.
For his continuous support, encouragement and advice throughout the entire study period, I am particularly indebted to Prof. Jhr. Dr. J.W. Wladimiroff.
I thank Prof. Dr. G.H. Zeilmaker for his critical remarks and advice during the discussions of this thesis.
I like to thank also Prof. Dr. A.C. Drogendijk and Prof. Dr. G. Hennemann for their willingness to be members of the committee.
The late Imprinetta van Gent, my teacher of gynaecologic ultrasonography, was co-author of several articles used in this thesis.
For their cooperation during the preparation of the articles, and for taking over a large share of my duties recently, I thank Bert Alberda, Henny Janssen-Caspers, Lenie van Os and Michael Scholtes, my colleagues of the IVF team.
Dr. F.H. de Jong gave valuable criticism on the studies on the value of plasma 17β-oestradiol determinations during IVF cycles.
Marien van der Stoep collected and combined many treatment and laboratory data for further analysis.
Dr. H.J.A. Schouten and Dr. Ir. P.I.M. Schmitz of the Department of Biostatistics gave statistical advice.
I thank the "Audiovisuele Dienst" and particularly Cor van Dijk for providing the figures for this thesis.
The articles could not have been written without the help and co-operation of the patients.
I am grateful to Organon Nederland B.V. and Medilog B.V./Kretz GmbH for their financial support.
I want to express my gratitude to my mother and my late father for allowing me to study medicine and for their support during that particular period.
I like to thank Prof. Dr. A.C. Drogendijk, Prof. Dr. H.C.S. Wallenburg, Prof. Jhr. Dr. J.W. Wladimiroff and Dr. H.F. Heins for giving me the opportunity to become a gynaecologist in their departments.
Finally, to Elly I want to express my gratitude for her support and patience, particularly during the completion of this manuscript.

CURRICULUM VITAE

1944	Born in Deventer
1957-1963	Haags Montessori Lyceum, The Hague, HBS-B
1963-1971	University of Utrecht, Medical School
1971	Board Certification Physician
1971-1980	General physician, The Hague
1980-1985	Residency Obstetrics and Gynaecology
1980-1981	Ikazia Hospital, Rotterdam (Dr.H.F. Heins)
1981-1984	Academic Hospital Rotterdam-Dijkzigt, Erasmus University Rotterdam (Prof.Dr.A.C. Drogendijk, Prof.Dr.H.C.S. Wallenburg, Prof.Jhr.Dr.J.W. Wladimiroff)
1984-1985	Ikazia Hospital, Rotterdam (Dr.H.F. Heins)
1985	Board Certification Obstetrics and Gynaecology

PRESENT APPOINTMENTS:

1985-	Academic Hospital Rotterdam Dijkzigt, Staff member of the Department of Obstetrics and Gynaecology