CLINICAL ASSISTED REPRODUCTION

Importance of Endometrial Quality in Women with Tubal Infertility During a Natural Menstrual Cycle for the Outcome of IVF Treatment

G. CSEMICZKY,^{1,3} H. WRAMSBY,¹ E. JOHANNISSON,² and B.-M. LANDGREN¹

Submitted: May 20, 1997

Accepted: September 8, 1997

Purpose: The importance of endometrial maturation at estimated time of implantation for the outcome of IVF treatment in regularly menstruating women with tubal infertility was evaluated.

Methods: FSH was measured on cycle day 3, on days 10–15 urine and blood were collected to estimate the day of the LH peak, and E_2 and P_4 were measured during the luteal phase, on cycle days 19–26. An endometrial biopsy was obtained on days LH + 3 to LH + 6.

Results: The number of subjects with delayed endometrial maturation was larger in the group of infertile women who did not become pregnant compared to pregnant women and controls. Those infertile women who did not become pregnant after IVF treatment also presented with a higher basal FSH on cycle day 3 and lower E_2 and P_4 AUC in the luteal phase. Six infertile women and two controls presented with mid- and late-proliferative endometrium in the luteal phase on cycle days LH + 3 to LH + 6, in the presence of adequate E_2 and P_4 secretion. Six morphological characteristics were compared in the three groups: (1) 17 infertile women who became pregnant, (2) 18 who did not become pregnant, and (3) 28 controls. The pregnant infertile women did not differ from the controls. The numbers of glandular and stromal mitoses were significantly higher in those women who did not become pregnant ($P < 0.01$) compared with those who became pregnant. Endometrial biopsies obtained on cycle

days LH + 5 and LH + 6 showed significant differences in glandular epithelial height ($P < 0.05$) and number of vacuolated cells among the nonpregnant women ($P < 0.01$), the pregnant women ($P < 0.05$), and controls.

Conclusions: A higher frequency of retarded endometrial development in women who did not become pregnant following IVF treatment was found. In some cases, endometrial insensitivity could most likely cause retarded endometrial development and failure of implantation after IVF treatment, which could not be overcome by routine luteal-phase support. However, our results do not allow conclusions concerning its relative importance compared to preembryo quality; this has to be investigated further.

KEY WORDS: endometrium; in vitro fertilization outcome; menstrual cycle; ovarian function; tubal infertility.

INTRODUCTION

The pregnancy rate in in vitro fertilization (IVF) is now 30–40% at most centers, and even if this is an improvement, attempts are being made to increase the implantation rate further. One possible cause for low implantation rate is suboptimal endometrial quality. During the normal menstrual cycle, the endometrium undergoes hormone-dependent proliferation and secretory differentiation in preparation for implantation.

Histologic procedures have been developed for characterization and evaluation of endometrial maturation in relation to circulating steroid hormone levels during the luteal phase of the menstrual cycle. The method of choice for estimation of endometrial quality is still that of Noyes *et al.* (1). Retarded endometrial development in the luteal phase has been suggested as a cause

¹ Department of Woman and Child Health, Division for Obstetrics & Gynecology, Karolinska Hospital, P.O. Box 140, S-171 76 Stockholm, Sweden.

² Laboratory of Analytical and Quantitative Cytology, Hôpital Cantonal, Geneva, Switzerland.

³ To whom correspondence should be addressed.

of implantation failure, and the prevalence among infertile women varies widely, from 12.4 to 65.3% (2–5). The incidence of endometrial retardation among fertile controls was less than 5% (6). This great variation in infertile women might, however, be due partly to inaccurate precision of histologic dating (7,8).

The aim of the present study was to evaluate the importance of endometrial maturation at estimated time of implantation for the outcome of IVF treatment in regularly menstruating women with tubal infertility.

MATERIALS AND METHODS

Study group

Forty-three women awaiting IVF treatment, who had failed to conceive for at least 2 years following tubal reconstructive surgery, volunteered for the study. All women were regularly menstruating and underwent, before surgery, a complete investigation for infertility including basal body temperature, characterization of the menstrual cycle by repeated serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), and progesterone (P_4) in the luteal phase. All fulfilled our standard criteria for normal ovulatory cycles (9). All men had normal spermograms on at least two occasions according to the World Health Organization (10) criteria. For clinical characteristics see Table I.

Control Group

During the same period, 30 apparently healthy women, with a history of regular periods and with proven fertility, volunteered for the study. None of them had used steroidal contraceptives or an intrauterine device during the previous 3 months. None had a history of pelvic inflammatory disease or an abortion

or delivery 1 year before admission to the study. For clinical characteristics see Table I.

Follicle Size and Endometrial Thickness Measurements

To determine the size of the dominant follicle, see on which day the follicle disappears, and measure the thickness of the endometrium, ultrasonographic investigations were performed transvaginally using a Siemens Sonoline SI/200 real-time scanner, equipped with a mechanical sector rotating 5–6.5-MHz transducer. All ultrasound measurements were made by the same person.

Study Plan

Blood samples were obtained from all subjects in a randomly selected cycle by venepuncture in the morning between 0730 and 0830. Five milliliters of blood was drawn each time into test tubes, then centrifuged within 30 min, and the serum was frozen and stored at -20°C until assayed. Blood was collected on days, 3, 10–15, and 19–26. On days 10–15 urine samples were collected to estimate the day of the LH peak, using a self-test (OvuQvick; Monoclonal Antibodies Inc., Sunnyvale, CA). The LH peak was also determined in serum samples. E_2 and P_4 were measured during the luteal phase, on cycle days 19–26. E_2 , P_4 , FSH, and LH measurements were performed with an Immulite system, DPC, LA, and chemiluminescence. E_2 and P_4 were analyzed with a competitive immunoassay. FSH and LH were analyzed with a noncompetitive immunoassay. Limits of determination were as follows: E_2 , 73–7340 pM; P_4 , 0.64–127 nM; FSH, 0.1–170 IU/L; and LH, 0.7–200 IU/L. Precision (within run) was as follows: E_2 , 6.3–15%; P_4 , 6.9–13%; FSH, 5.4–7.7%; and LH, 4.8–6.5%.

In the luteal phase P_4 and E_2 concentrations were expressed as areas under the curve (AUC), which were calculated as sums of partial areas obtained according to the following formula

$$\begin{aligned} \text{area} = & \text{time} \times [(\text{level a} + \text{level b})/2] \\ & + \text{time} \times [(\text{level b} + \text{level c})/2] \\ & + \text{time} \times [(\text{level c} + \text{level d})/2] \\ & + \text{time} \times [(\text{level d} + \text{level e})/2] \end{aligned}$$

In the same menstrual cycle, endometrial biopsies were obtained from the anterior wall of the uterine cavity using a Randall curette (Stille Werner AB, Stockholm, Sweden) without cervical dilatation or

Table I. Certain Characteristics of 43 Infertile Women (25 of Whom Did Not and 18 of Whom Did Become Pregnant After IVF Treatment) and 30 Controls (Mean $\pm$ SD)

	Pregnant	Nonpregnant	Controls
<i>n</i>	18	25	30
Age (yr)	32.9 $\pm$ 1.8	33.4 $\pm$ 2.1	33.8 $\pm$ 2.5
Height (cm)	167 $\pm$ 4.9	168 $\pm$ 5.8	167 $\pm$ 5.3
Weight (kg)	63.8 $\pm$ 4.3	63.4 $\pm$ 6.2	63.2 $\pm$ 8.0
BMI (kg/m ²)	22.5 $\pm$ 3.4	22.7 $\pm$ 2.6	22.6 $\pm$ 2.7
Cycle length (days)	28.2 $\pm$ 2.6	28.4 $\pm$ 4.7	28.5 $\pm$ 4.8

anesthesia. The criteria of Noyes *et al.* (1) were used for the histologic dating. The day of biopsy was grouped in relation to the LH peak and varied from LH + 3 to LH + 6. Endometrium out of phase was defined as histologic dating differing by more than 2 days from that in relation to the LH peak.

The specimens were fixed immediately in Bouin's solution for light microscopic examination after paraffin embedding and hematoxylin-eosin staining. Ten arbitrarily chosen microscopic fields were analyzed in each biopsy specimen. (Zeiss light microscope, $\times 10$, wide-angle eyepieces with micrometer disk, objective $\times 40$; Carl Zeiss, Germany). In each microscopic field the following six indices were assessed: (i) number of transversely sectioned glands (expressed as glands per microscopic field), (ii) number of glandular mitoses (expressed as mitoses per 1000 glandular cells), (iii) number of stromal mitoses (expressed as mitoses per 1000 stromal cells), (iv) diameter (μm) of the glandular lumen in the transversely sectioned glands, (v) height (μm) of the glandular epithelium, and (vi) number of glandular cells with basal or subnuclear vacuolation (expressed as basal vacuolated cells per 1000 glandular cells). The morphological evaluation of the endometrial biopsies was performed blindly by one of the authors as described previously (11).

IVF Treatment Protocol

All patients followed our standard long gonadotropin releasing hormone agonist (GnRHa) and human menopausal gonadotropin (hMG) protocol for stimulation as described (12). GnRHa (Suprefact; Svenska Hoechst AB, Stockholm, Sweden) was administered as a nasal spray, $6 \times 200 \mu\text{g}$ daily, starting on day 21 of the menstrual cycle. After down-regulation was verified by ultrasound examination, hMG (Pergonal; Serono Nordic AB, Sollentuna, Sweden) was administered i.m., 150–225 IU daily. Follicular development was monitored by vaginal ultrasound measurements of follicles and endometrium using a Siemens Sonoline SI/200 combined with blood samples for E_2 analysis. The day for ovum pickup was determined when the leading follicle reached a diameter of 17 mm and the serum levels of E_2 reached at least 2500 pM. Then 5000 IU human chorionic gonadotropin (hCG; Profasi; Serono Nordic AB) was given i.m. Approximately 35 hr later, ovum pickup was performed by transvaginal ultrasound-guided follicle aspiration. Gamete and preembryo handling as well as embryo replacement has been described previously (12). Luteal-phase support was given using 5000 IE hCG i.m., at 3, 6, and 9 days

after embryo replacement. During the study period, treatment was restricted to three cycles (two women in the nonpregnant group had four cycle starts but only three treatment cycles) and the number of replaced preembryos was limited to three.

Statistical Methods

Results are presented as means $\pm$ SD, medians, and ranges (Table IV) except where indicated otherwise. Statistical evaluation was performed using ANOVA, Student's *t* test, and Fishers' exact test whenever appropriate. Comparison of the hormonal characteristics between the groups was performed by using Mann-Whitney two-tailed test. For statistical evaluation of the morphometric indices related to the midcycle LH surge, ANOVA was used. *P* values < 0.05 were considered significant.

Ethical Aspects

The study was approved by the Ethics Committee of the Karolinska Hospital.

RESULTS

Clinical characteristics of the study group and the controls are presented in Table I. Certain characteristics of the infertile women with respect to outcome of IVF treatment are shown in Table II. Those women who became pregnant had significantly fewer treatment cycles ($P < 0.001$) and needed fewer ampoules of gonadotropins ($P < 0.001$). They also had fewer oocyte retrievals ($P < 0.001$) and more oocytes per retrieval ($P < 0.001$), a higher fertilization rate ($P < 0.01$), and more replaced preembryos per replacement ($P < 0.01$) compared with nonpregnant women.

Six infertile women and two controls (Table III) did have mid- and late-proliferative endometrium in the luteal phase on cycle days LH + 3 to LH + 6. One infertile woman and one control did present with excellent ovarian function (low FSH and high E_2 and P_4 AUC, subject no. I-2 and C-1) (13). The others exhibited impaired ovarian function as indicated by the high basic FSH and low P_4 AUC in the luteal phase. These eight women were excluded from further analyses.

Table IV shows E_2 and P_4 AUC in the luteal phase in 37 infertile women and 28 controls in relation to endometrial maturation. Normal controls did not differ from the infertile women who became pregnant. The endometrial dating resulted in significantly more cases

Table II. Outcome of IVF Treatment in 43 Infertile Women

Characteristics	Pregnant	Nonpregnant	Total
	18	25	43
Started cycles (per patient)	36 (2.0)	78 (3.1)**	114 (2.7)
No. of ampoules of gonadotropins per cycles	28.1 $\pm$ 2.3	31.8 $\pm$ 4.1**	30.3 $\pm$ 3.9
No. of oocyte retrievals (%)	36 (100)	58 (74)	94 (82)
No. of oocytes retrieved (per retrieval)	163 (4.5)	153 (2.6)**	316 (3.4)
No. of fertilized oocytes (%)	129 (79)	106 (69)*	235 (74)
No. of ET (%)	35 (97)	42 (54)*	77 (68)
No. of preembryos replaced (per ET)	99 (2.8)	87 (2.1)*	186 (2.4)
No. of pregnancies per ET (%)	18	0	18 (23)
Implantation index ^a	0.20	0	0.11
No. of live births per patient (%) ^b	15 (83)	0	15 (35)

^a No. of implantations per no. of preembryos replaced.^b Two twin pregnancies.* $P < 0.01$ Student's t test and Fisher's exact test.** $P < 0.001$ Student's t test and Fisher's exact test.**Table III.** Certain Characteristics and Outcome of IVF Treatment in Six Infertile Women and Two Controls with Proliferative Endometrium Obtained in the Luteal Phase

Subject No.	Age (yr)	BMI (kg/m ²)	FSH (IU/L) on cycle day 3	E ₂ AUC (pM) × days	P ₄ AUC (nM) × days	P ₄ (nMol/l)			Biopsy day related to LH peak (day 0)	Endometrial dating (1)	Outcome of IVF treatment
						LH + 3–LH + 4	LH + 6–LH + 7	LH + 9–LH + 10			
Infertile											
1-1	33	21.3	16.5	3,289	94.1	18.9	16.1	12.8	+3	Mid prolif.	Nonpregnant
1-2	35	21.6	7.7	3,564	255.0	25.8	30.9	25.4	+6	Mid prolif.	Nonpregnant
1-3	36	20.8	19.0	1,746	103.7	16.8	17.9	17.2	+4	Late prolif.	Nonpregnant
1-4	37	19.3	16.1	3,089	89.6	6.6	13.4	16.4	+4	Late prolif.	Nonpregnant
1-5	36	22.3	13.5	3,353	59.3	14.1	12.8	8.8	+6	Late prolif.	Nonpregnant
1-6	34	22.2	37.1	2,885	85.3	19.96	18.6	12.8	+6	Late prolif.	Pregnant (sp. abortion)
Controls											
C-1	32	22.4	1.7	15,690	783.5	65.9	99.5	48.6	+6	Late prolif.	—
C-2	36	22.5	16.4	1,690	83.5	12.3	16.1	11.3	+4	Late prolif.	—

of retarded endometrial development ($n = 12$) in the infertile women who did not become pregnant compared to those who became pregnant ($r = 3$) ($P <$

0.01). A comparison of six morphological characteristics of the endometrium in 37 women before IVF treatment and 28 controls is shown in Table V. The biopsies

Table IV. E₂ and P₄ AUC in the Luteal Phase in Relation to the Endometrial Maturation and the Outcome of IVF Treatment in 37 Infertile Women Medians and Ranges

	Pregnant	Nonpregnant	Statistical analysis: nonpregnant vs pregnant ^a
Endometrium in phase	$n = 15$	$n = 7$	
E ₂ AUC (pM $\times$ days) in luteal phase	7,573 (5,341–11,740)	4,795 (1,054–7,342)	**
P ₄ AUC (nM $\times$ days) in luteal phase	538 (259–1,123)	352 (190–462)	*
Endometrium out of phase	$n = 3$	$n = 12$	
E ₂ AUC (pM $\times$ days) in luteal phase	6,813 (4,334–8,946)	4,184 (1,746–7,161)	**
P ₄ AUC (nM $\times$ days) in luteal phase	412 (86–544)	277 (59–451)	*

^a Mann-Whitney test: * $P < 0.05$; ** $P < 0.01$.

Table V. Morphometric Analyses of Various Indices of the Endometrium in 37 Infertile Women and 28 Controls, Related to the Outcome of IVF Treatment (Means $\pm$ SD)

	Days of the luteal phase related to LH peak (0)										Statistical analysis ^a			
	LH + 3 and LH + 4				LH + 5 and LH + 6				LH + 3 and LH + 4		LH + 5 and LH + 6			
	Infertile		Controls		Infertile		Controls		Controls vs pregnant nonpregnant		Controls vs pregnant nonpregnant			
	Pregnant ^b	Nonpregnant	Pregnant	Nonpregnant	Pregnant	Nonpregnant	Pregnant	Nonpregnant	Controls vs pregnant nonpregnant	Pregnant vs nonpregnant	Controls vs pregnant nonpregnant	Pregnant vs nonpregnant	Controls vs pregnant nonpregnant	Pregnant vs nonpregnant
<i>n</i>	7	9	16	11	10	12								
Number of glands/mm ²	22.8 ($\pm$ 3.3)	202.2 ($\pm$ 1.4)	23.7($\pm$ 2.4)	24.6 ($\pm$ 2.9)	22.7 ($\pm$ 4.1)	25.8 ($\pm$ 5.7)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Glandular mitoses per 1000														
glandular cells (<i>n</i>)	12.6 ($\pm$ 2.4)	16.5 ($\pm$ 4.2)	11.5 ($\pm$ 0.5)	2.3 ($\pm$ 1.2)	4.6 ($\pm$ 4.9)	0.4 ($\pm$ 0.4)	N.S.	N.S.	**	*	N.S.	**	*	*
Stromal mitoses per 1000 stromal cells (<i>n</i>)	1.2 ($\pm$ 0.4)	3.8 ($\pm$ 2.4)	0.6 ($\pm$ 0.4)	0.9 ($\pm$ 0.2)	2.4 ($\pm$ 1.4)	0.18 ($\pm$ 0.3)	N.S.	N.S.	**	*	N.S.	**	*	*
Glandular diameter (μ m)	48.2 ($\pm$ 5.7)	54.4 ($\pm$ 5.4)	53.4 ($\pm$ 4.6)	58.9 ($\pm$ 5.3)	57.3 ($\pm$ 2.4)	63.3 ($\pm$ 4.2)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Glandular epithelial height (μ m)	23.4 ($\pm$ 0.3)	23.5 ($\pm$ 0.6)	23.3 (0.9)	18.4 ($\pm$ 0.5)	21.6 ($\pm$ 1.2)	16.6 ($\pm$ 3.6)	N.S.	N.S.	N.S.	N.S.	N.S.	**	**	N.S.
Vacuolated cells per 1000 glandular cells (<i>n</i>)	342.5 ($\pm$ 82.4)	329.3 ($\pm$ 77.6)	325.6 ($\pm$ 57.6)	99.0 ($\pm$ 5.0)	115.4 ($\pm$ 33.7)	78.8 ($\pm$ 8.3)	N.S.	N.S.	N.S.	N.S.	*	**	**	N.S.

^a ANOVA: **P* < 0.05; ***P* < 0.01.^b IVF outcome.

were taken on cycle days LH +3 and +4 and days LH +5 and +6. The pregnant women in the infertile group did not differ from the controls. The numbers of glandular mitoses and stromal mitoses were significantly higher in those women who did not become pregnant ($P < 0.01$) compared with those who became pregnant, independent of the cycle days. The endometrial biopsies obtained on cycle days LH + 5 and LH + 6 showed significant differences in glandular epithelial height ($P < 0.01$) and number of vacuolated cells among the nonpregnant ($P < 0.01$), the pregnant ($P < 0.05$), and the control women.

DISCUSSION

In our study delayed endometrial maturation in the luteal phase was more common in women who did not become pregnant after IVF, compared both to controls and to those women who became pregnant, indicating that the endometrium in this population was significant for implantation. Six infertile women and two controls presented with mid- and late-proliferative endometrium in the luteal phase on cycle days LH + 3 to LH + 6, in the presence of E_2 and P_4 secretion (11). Our observation of two fertile control women having proliferative endometrium in the luteal phase indicates that fertile women accidentally may have serious endometrial disturbances. Our findings are in agreement with Li *et al.* (5), who found normal P_4 levels in at least 20% of infertile women in combination with proliferative endometrium and suggested end organ insensitivity as explanation in these cases. In another study, Li *et al.* (14) showed some morphological similarities in the endometrium between women with unexplained infertility and fertile women treated with P_4 receptor antagonist, which strongly supports this theory.

The importance of endometrial adequacy for implantation of the preembryo and establishment and maintenance of pregnancy is still a matter of discussion. Satisfactory endometrial development depends on normal amounts of the steroid hormones E_2 and P_4 produced by the ovary (15–17). There have been many attempts to determine the serum P_4 levels that will indicate normal endometrial development during the luteal phase. Israel *et al.* (18) reported that a midluteal serum P_4 of 10 nM or higher was indicative of ovulation and of the maturation of a normal secretory endometrium. Several other investigators confirmed correlation between endometrial morphology and the presence of P_4 secretion in the luteal phase (7,19,20).

The steroid hormones mediate their effects on the target tissue, the endometrium, via cytosolic receptors. Adequacy of the luteal phase can therefore be assessed only by both multiple P_4 determinations and endometrial biopsies (3,21,22).

Data available from oocyte donation programs suggest that the postmenopausal endometrium maintains its ability to respond to exogenous steroids and that it has the capacity to develop into a receptive endometrium for preembryo implantation and gestation (23–26). Embryo quality is known to be a major contributor to the failure or the success of IVF treatment and the age of the women donating oocytes has been shown to have higher significance for the outcome of treatment than the age of the recipient (27,28). Moreover, the women who did not become pregnant in our study presented with lower P_4 and E_2 secretion in the luteal phase compared with pregnant women, which could obviously not be overcome by ovarian stimulation and luteal-phase support. This finding indicates that ovarian function may be more important for the outcome of IVF treatment than endometrial maturation.

In conclusion, we found a higher frequency of retarded endometrial development in women who did not become pregnant following IVF treatment. However this may be a secondary effect due to impaired ovarian function in these women, as judged from the significantly lower E_2 and P_4 . In some cases, endometrial insensitivity might be involved in retarded endometrial development and failure of implantation after IVF treatment. However, our results do not allow conclusions concerning the relative importance of the endometrium compared to preembryo quality (29–31). This is a matter that has to be investigated further.

ACKNOWLEDGMENTS

This work was funded by the Medical Research Council (K97-19X-12238-01A), the Karolinska Institute, and Karolinska Hospital. The authors thank Dr. Gabriel Fried for the helpful suggestions and critical reading of the manuscript.

REFERENCES

1. Noyes RW, Hertig AT, Rock J: Dating the endometrial biopsy. *Fertil Steril* 1950;1:3–25
2. Jones GS: Some newer aspects of management of infertility. *JAMA* 1949;141:1123
3. Wentz AC: Endometrial biopsy in the evaluation of infertility. *Fertil Steril* 1980;33:121–124

4. Balasch J, Creus M, Marquez M, Bruzaco I, Vanrell JA: The significance of luteal phase deficiency on fertility: A diagnostic and therapeutic approach. *Hum Reprod* 1986;1:145-147
5. Li TC, Dockery P, Cooke ID: Endometrial development in the luteal phase of women with various types of infertility: Comparison with women of normal fertility. *Hum Reprod* 1991;6:325-330
6. Li TC, Dockery P, Rogers AW, Cooke ID: A quantitative study of endometrial development in the luteal phase: Comparison between women with unexplained infertility and normal fertility. *Br J Obstet Gynaecol* 1990;97:576-582
7. Johannisson E, Parker RA, Landgren B-M, Diczfalussy E: Morphometric analysis of the human endometrium in relation to peripheral hormone levels. *Fertil Steril* 1982;38:564-571
8. Li TC, Rogers AW, Dockery P, Lenton E, Cook ID: A new method of histologic dating of human endometrium in the luteal phase. *Fertil Steril* 1988;50:52-60
9. Landgren B-M, Undén AL, Diczfalussy E: Hormonal profile of the cycle in 68 normally menstruating women. *Acta Endocrinol (Kbh)* 1980;94:89-98
10. WHO: Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interactions. Cambridge, WHO, 1987, pp 9-11
11. Johannisson E, Landgren B-M, Rohr HP, Diczfalussy E: Endometrial morphology and peripheral hormone levels in women with regular menstrual cycles. *Fertil Steril* 1987;48:401-408
12. Csemiczky G, Hagenfeldt K, Wramsby H: Selection of ovarian stimulation protocol is related to IVF treatment outcome in women 35 years of age and older. *J Assist Reprod Genet* 1994;11:474-477
13. Csemiczky G, Wramsby H, Landgren B-M: Ovarian function in relation to the outcome of IVF treatment in regularly menstruating women with tubal infertility. *J Assist Reprod Genet* 1995;12:683-688
14. Li TC, Dockery P, Thomas P, Rogers AW, Lenton E, Cook ID: The effect of progesterone receptor blockade in the luteal phase of normal fertile women. *Fertil Steril* 1988;50:732-742
15. Gordon M, Kohorn E, Gore B, Rice S: Effect of postovulatory oestrogens on the fine structure of the epithelial cells in the human endometrium. *J Reprod Fertil* 1973;34:375-378
16. Wynn R: Histology and structure of the human endometrium. In *Biology of the Uterus*, RM Wynn (ed) New York, London, Plenum Press, 1977, pp 341-376
17. Cornille FJ, Lauweryns JM, Brosens IA: Normal human endometrium. *Gynecol Obstet Invest* 1985;20:113-129
18. Israel R, Mishell DJ, Stone S, Thorneycroft I, Moyer D: Single luteal phase progesterone assay as an indicator of ovulation. *Am J Obstet Gynecol* 1972;112:1043
19. Radwanska E, Swyer GM: Plasma progesterone estimation in infertile women and in women under treatment with clomiphene and chorionic gonadotropin. *Br J Obstet Gynecol* 1974;81:107-112
20. Li TC, Lenton E, Dockery P, Rogers AW, Cook ID: The relation between daily salivary progesterone profile and endometrial development in the luteal phase of fertile and infertile women. *Br J Obstet Gynecol* 1989;96:445-453
21. Cooke ID, Morgan CA, Parry TE: Correlation of endometrial biopsy and plasma progesterone levels in women. *J Obstet Gynecol Br Commonwealth* 1972;78:647-650
22. Cumming DC, Honore LH, Scott JZ, Williams KP: The luteal phase in infertile women: comparison of simultaneous endometrial biopsy and progesterone levels. *Fertil Steril* 1985;43:715-719
23. Lutjen P, Trounson A, Leeton J, Findlay J, Wood C, Renou P: The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. *Nature* 1984;307:174-175
24. Rosenwaks Z: Donor eggs: Their application in modern reproductive technologies. *Fertil Steril* 1987;47:895-909
25. Drosch K, Navot D, Scott R, Kreiner D, Liu H, Rosenwaks Z: Transdermal estrogen replacement in ovarian failure for ovum donation. *Fertil Steril* 1988;50:931-934
26. Navot D, Bergh P, Williams M, Garrisi G, Guzman I, Sandler B, Fox J, Schreiner-Engel P, Hofmann G, Grunfeld L: An insight into early reproductive processes through the in vivo model of ovum donation. *J Clin Endocrinol Metab* 1991;72:408-414
27. Meldrum D: Female reproductive aging-ovarian and uterine factors. *Fertil Steril* 1993;59:1-5
28. Navot D, Drews M, Bergh P, Guzman I, Karstaedt A, Scott R, Garrisi G, Hoffman G: Age related decline in female fertility is not due to diminished capacity of the uterus to sustain embryo implantation. *Fertil Steril* 1994;61:97-100
29. Erenus M, Erenus M, Zouves C, Rajamahendran P, Leung S, Fluker M, Gomel V: The effect of embryo quality on subsequent pregnancy rates after in vitro fertilization. *Fertil Steril* 1991;56:707-710
30. Steer C, Mills C, Tan SL, Campbell S, Edwards R: The cumulative embryo score: A predictive embryo scoring technique to select the optimal number of embryos to transfer in an in vitro fertilization and embryo transfer program. *Hum Reprod* 1992;7:117-119
31. Gioretti C, Terrou P, Auquier P, Hans E, Spach J-L, Salzman J, Roulier R: Embryo score to predict implantation after in vitro fertilization: based on 957 single embryo transfers. *Hum Reprod* 1995;10:2427-2431