Prediction of Pregnancy Rate of In Vitro Fertilization and Embryo Transfer in Women Aged 40 and over with Basal Uterine Artery Pulsatility Index

CHI-HSIN CHIANG,[1] T'SANG-T'ANG HSIEH,[1] MING-YANG CHANG,[1,2] CHII-SHINN SHIAU,[1] HUNG-CHIH HOU,[1] JENN-JEIH HSU,[1] and YUNG-KUEI SOONG[1]

Submitted: January 14, 2000
Accepted: March 9, 2000

Purpose: *The purpose was to determine the effect of basal uterine perfusion on the pregnancy rates of in vitro fertilization and embryo transfer (IVF-ET) in women aged 40 and above.*
Methods: *A total of 47 patient aged 40 and over underwent IVF-ET. The conception cycles and the nonconception cycles were compared.*
Results: *Of the 47 patients, 4 patients were pregnant (8.5%). The mean age, basal follicle stimulating hormone (FSH), basal estradiol (E_2) level, antral follicle count (AFC), number of ampoules of gonadotropin used, E_2 levels and endometrial thickness on the day of human chorionic gonadotropin (hCG) administration, number of retrieved and fertilized oocytes, and number of transferred embryos were not statistically significant between the conception and nonconception cycles. However, the basal uterine artery pulsatility index (UA PI) was significantly lower in the conception cycles ($P < 0.001$). The receiver operating characteristics (ROC) curve analysis for basal FSH, AFC, and basal UA PI in predicting the pregnancy rate of IVF in patients aged ≥ 40 were demonstrated. The best prediction rate was achieved by a pulsatility index cutoff of < 2.0 for a receptive uterus.*
Conclusions: *Increased uterine perfusion in the early follicular phase enhanced the pregnancy rate of IVF in women aged 40 and above. It is therefore essential that patients aged ≥ 40 with poor basal uterine perfusion should be identified early in the early follicular phase of the menstrual cycle to apply appropriate intervention to improve the uterine circulation for the subsequent chance of pregnancy.*

KEY WORDS: Basal uterine perfusion; color Doppler ultrasound; IVF-ET, pregnancy rate; women aged 40 and above.

[1] Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, 199, Tung Hwa North Road, Taipei 10591, Taiwan, Republic of China.
[2] To whom correspondence should be addressed.

INTRODUCTION

Advancing female age is believed to have adverse effects on the outcome of assisted conception cycles. In the literature, women aged 40 and above exhibited significant differences in reduction of number of antral follicles, oocytes or embryos (1–3), poor ovarian response to exogenous gonadotropins (4), lower chance of pregnancy and higher chance of spontaneous abortion in in vitro fertilization (IVF) (3,5,6). The number of follicles decreased between 22 and 42 years of age, decreasing especially rapidly after the age of 38 (1,7). In addition to the decrease in the number of oocytes, folliculogenesis also is compromised to yield more poor-quality oocytes in the reproductively aged ovary due to genetic degeneration within oocytes (8,9). Similarly, the effect of age on the uterus may include reduced vascular perfusion (10). Accordingly, impaired uterine blood flow has been suggested as a possible cause of infertility (10). Recently, the association of a lower preovulatory uterine artery pulsatility index (UA PI) with a higher pregnancy rate and implantation rate in IVF has been established by using transvaginal color Doppler sonography (TV-CDS) (11–13). Moreover, the use of low-dose aspirin treatment in the early follicular phase to increase the basal and subsequent uterine perfusion was reported to have a higher pregnancy rate (11). This suggests that the subsequently better endometrial receptivity may be related to a higher grade of uterine perfusion in the early follicular phase.

Despite awareness of good uterine blood flow in the periovulatory stage for successful assisted conception, little information is available regarding the uterine perfusion in the early follicular phase or basal uterine perfusion on the pregnancy rate of IVF among women aged 40 and above. Although basal follicle-stimulating

1058-0468/00/0900-0409$18.00/0

hormone (FSH) and antral follicle count (AFC) have been reported to be predictive on the outcome of IVF (14–16), the optimal parameter in predicting the pregnancy rate of IVF in women aged 40 and above is currently absent. The aim of this study was to determine the effect of basal uterine perfusion on the pregnancy rate of IVF in women aged 40 and above and compared it with aforementioned predictive markers with receiver operating characteristics curve.

MATERIALS AND METHODS

From December 1997 to December 1998, a total of 58 patient aged 40 and over were scheduled to undergo IVF at our IVF unit. Of these, 11 patients were cancelled due to poor ovarian response. Forty-seven patients aged 40 and over undergoing IVF-ET were enrolled. This study was approved by the institutional review board at Chang Gung Memorial Hospital. Hysteroscopy was routinely performed on all patients to confirm a normal uterine cavity in the preceding menstrual cycles before IVF.

Ultrasound evaluation was carried out routinely for all patients on day 3 of the cycle between 0700 to 0900 using an Acuson computed ultrasound 120XP/10 (Acuson Inc., Mountain View, CA) by one operator (CHC). A 7-MHz high-frequency transducer vaginal sector scanner for imaging with a 5-MHz pulsed Doppler system for blood flow analysis was used. The spatial peak temporal average intensity of ultrasound for B-mode and Doppler examinations was below 50 mW/cm^2. First, both ovaries were scanned carefully and total antral follicles (2–8 mm) were counted (16). Then, the color signal for the main uterine artery was sought paracervically. Both sides of uterine artery PI were measured during each scan. Maximum waveform amplitude was obtained by moving the angle of the transducer. The lowest PI value for each patient was used for statistical analyses. The intraobserver coefficients of variability for all the measurements of PI was $<$ 12%. Each examination lasted approximately 20 min.

All patients received leuplide acetate (1 mg/day subcutaneously) (Lupron; Abbot Australia Pty, Ltd, Kurnell, New South Wales, Australia) 10 days before the expected day of menstruation (long protocol) or on day 2 of menstruation (short protocol). This was continued through the treatment cycle until the time of human chorionic gonadotrophin (hCG) administration. Two ampoules of FSH (metrodin; Serono Laboratories, Randolph, MA) and 2 ampoules of human menopausal gonadotropin (hMG) (Pergonal; Serono Laboratories) were administered daily for the first 3 days. Then, the dose was tailored according to the number and sizes of follicles as monitored daily using transvaginal ultrasound and serial serum estradiol levels. Ten thousand international units of hCG (Pregnyl; Organon, Oss, the Netherlands) were administered intramuscularly when the mean diameter of two or more leading follicles reached 16 mm. Transvaginal ovum retrieval was scheduled 36 hr later. Embryo transfer was performed 48–72 hr following fertilization. Luteal phase support was supplied for all patients in the form of oral progesterone (Utrogestan, Laboratories Piette International, Brussels, Belgium) and hCG (Pregnyl) injections, 2500 IU intramascularly every fourth day starting on the day of embryo transfer and continued for 2 weeks. A urine pregnancy test was performed 17 days after the transfer procedure. Clinical pregnancy was defined as the presence of at least one intrauterine gestational sac on transvaginal sonography or pathological confirmation of gestational tissue from surgical procedures.

Parameters determined on all patients included age, day 3 FSH, day 3 estradiol (E_2), antral follicles count, basal uterine artery PI, number of ampoules of gonadotropins used, E_2 level and endometrial thickness (mm) on day of hCG administration, number of dominant follicles, number of retrieved and fertilized oocytes, number of embryos transferred, and clinical pregnancy rate. The conception and nonconception cycles were compared.

Results are presented as mean values $\pm$ standard deviation (SD). Comparisons between means among the study groups were performed using *t* test. A value of $P < 0.05$ was considered statistically significant. Receiver operating characteristics (ROC) curve (GraphROC for windows, Turku, Finland) and reliability tests were used. Areas under the ROC curve with standard error were shown.

RESULTS

Of the 47 patients aged 40 and over undergoing IVF, 4 patients were pregnant. The pregnancy rate was 8.5%. The mean age, basal FSH, basal E_2 level, and AFC were not statistically significant between the conception and nonconception cycles (Table I). There was no statistically significant difference in the number of ampoules of gonadotropin used, E_2 levels and endometrial thickness on the day of hCG administration, and number of dominant follicles between both groups (Table I). There was no difference in the number of

Table I Cycle Profile of the IVF Program Between the Pregnant Group and the Nonpregnant Group of Women Aged 40 and over[a]

Variable	Women aged 40 and over undergoing IVF		P
	Nonpregnant ($n = 43$)	Pregnant ($n = 4$)	
Age (year)	41.8 ± 2.0	42.0 ± 0.7	NS[b]
Day 3 FSH (mIU/ml)	6.9 ± 2.8	5.9 ± 0.6	NS
Day 3 E_2 level (pg/ml)	49.5 ± 31.3	40.7 ± 15.8	NS
No. of antral follicles (2–8 mm)	7.8 ± 3.8	9.5 ± 3.0	NS
Basal uterine artery PI	2.6 ± 0.5	1.9 ± 0.2	< 0.001
No. of ampoules of gonadotropins used	47.7 ± 12.8	43.5 ± 5.1	NS
E_2 levels on the day of hCG used (pg/ml)	1551.0 ± 907.0	1714.0 ± 878.0	NS
Endometrial thickness (mm) on the day of hCG administration	11.2 ± 2.3	10.4 ± 1.1	NS
No. of dominant follicles	8.0 ± 2.5	9.0 ± 3.5	NS
No. of retrieved oocytes	6.5 ± 3.7	6.8 ± 2.0	NS
No. of fertilized oocytes	5.4 ± 2.4	5.7 ± 1.2	NS
No. of transferred embryos	4.5 ± 1.9	4.0 ± 0.5	NS
Pregnancy rate (%)	0/47 (0%)	4/47 (8.5%)	—

[a] Data are mean ± SD.
[b] Not significant.

retrieved and fertilized oocytes and number of transferred embryos in either group (Table I). However, the basal UA PI was significantly lower in the conception cycles ($P < 0.001$) (Table I).

The use of basal UA PI in predicting the pregnancy rate of IVF in patients aged ≥ 40 was superior to that using basal FSH or AFC in the ROC curve analysis (Fig. 1). Areas under the ROC curve for UA PI, basal FSH, and AFC were 0.9070 ± 0.0000, 0.6395 ± 0.1214, and 0.6337 ± 0.1810, respectively (Fig. 1). The predictive values of different pulsatility index cutoffs for a receptive uterus are given in Table II. The best prediction rate was achieved by a pulsatility index cutoff of < 2.0 with a sensitivity of 75%, specificity of 84%, positive predict rate of 30%, and negative predictive rate of 97% for a receptive uterus. The efficacy was 83% (Table II).

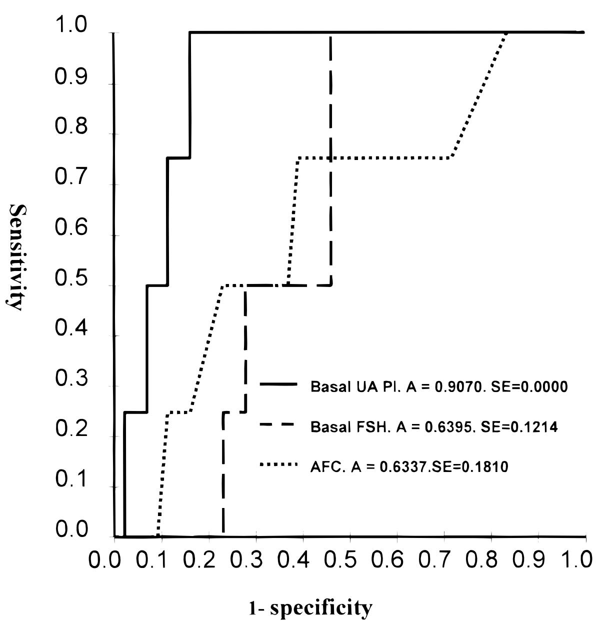

Fig. 1. Receiver operating characteristics (ROC) curve of the basal uterine artery pulsitility index (basal UA PI), antral follicle count (AFC), and basal follicle-stimulating hormone (basal FSH). A, The area under the ROC curve; SE, standard error.

DISCUSSION

Advancing age has been shown to have adverse effects on female fertility due to tubal disease from pelvic infection, deciliation of the fallopian tubal endothelium, ovulatory dysfunction, endometriosis and fibroids, and reduced vascular perfusion of the uterus (17). In addition, there also is an increased risk of decreasing retrieved oocytes, transferred embryos, and pregnancy rate and increasing cancellation rate and spontaneous abortion rate in assisted reproduction in women aged 40 and above (2–6,17). Moreover, the pregnancy rate of assisted reproductive technologies

Table II. Predictive Values of Basal Uterine Artery Pulsatility Index (UA PI) Cutoffs for a "Receptive" Uterus in IVF Patients Aged ≥ 40

	Sensitivity	Specificity	PPV[a]	NPV[a]
UA PI < 2.0	75%	84%	30%	97%
UA PI < 2.5	100%	58%	18%	100%
UA PI < 3.0	100%	19%	10%	100%

[a] PPV, Positive predictive values; NPV, negative predictive values.

in women aged 40 and above ranges only from 6.1 to 9.5% (17). Despite the poor pregnancy rate, there currently is no optimal predictive marker to predict the pregnancy rate of IVF in women aged 40 and above. In the present study, we have studied the relevance of uterine artery impedance in the early follicular phase in predicting the pregnancy rate of IVF in women aged 40 and above.

The assessment of uterine artery circulation on the day of hCG administration or following oocyte retrieval has been suggested as a predictive marker of pregnancy rate among women undergoing IVF treatment (11–13). Steer et al. (12) have suggested that a pulsatility index > 3.0 on the day of ET indicated a particularly low uterine receptivity. Cacciatore et al. (13) found implantation becoming very unlikely when pulsatility index was > 3.3 and resistance index $>$ 0.95 or when no velocities were detected at the end of the diastole of the uterine artery. Coulam and co-workers (18) also found a significant decrease of pregnancy rate when uterine artery pulsatility index was > 3.3 before ET. However, a prospective, randomized, double-blind, and placebo-controlled study showed that low-dose aspirin treatment started on the 21st day of the preceding menstrual cycle significantly improved the ovarian responsiveness, uterine and ovarian blood flow velocity on the day of HCG administration, implantation, and pregnancy rates in IVF patients (19). Wada et al. (11) showed that aspirin in low daily amount, started from day 1 together with hormone replace therapies (HRT) was associated with improved uterine blood flow and satisfactory pregnancy rate. These findings suggested the importance of improved basal uterine perfusion, essentialness of evaluating uterine artery pulsatility indices in the early follicular phase, and correlation between basal uterine perfusion and pregnancy rate of IVF. The present study showed that pregnant IVF patients aged 40–45 years presented lower uterine artery pulsatility index (mean $= 1.9 \pm 0.2$) in the early follicular phase compared with the nonconception cycles (mean $= 2.6 \pm 0.5$). The presence of lower basal uterine artery pulsatility index may indicate a more prominent uterine perfusion in the early follicular phase and subsequently might have a higher grade of uterine perfusion during ovarian stimulation. As a result, a better endocrine, paracrine, and autocrine milieu within the uterus may be present in the early follicular phase and during superovulation. Hence, a better subsequent uterine receptivity or higher chance of implantation may be expected in woman aged 40 and above with lower basal uterine artery pulsatililty index.

One might speculate that basal E_2 levels may play a role in influencing the basal uterine artery pulsatility index because several studies have shown the relationships between the effect of estrogen and uterine blood flow, while other studies were contradictive (12,13,20–22). de Ziegler et al. (20) indicated that E_2 at physiological levels decreases vascular resistance of uterine arteries in young women with absent or nonfunctioning ovaries. Hillard et al. (21) also reported that HRT (transdermal E_2, 0.05 mg/day) has a profound effect on arterial tone in postmenopausal women. Moreover, Cacciatore and co-workers (13) found that E_2 levels at the moment of ET were correlated with uterine artery pulsatility indices. Besides, uterine artery pulsatility index on the date of ET also correlated with the number of retrieved oocytes (13). However, Steer et al. (12) did not find any correlation between E_2 and uterine artery pulsatility index in their IVF patients before the time of ET. In addition, high E_2 levels during and after the 10th week of gestation might be associated with decreased uterine blood flow volume (22). In the present study, the lower uterine artery pulsatility index was not secondary to the elevation of basal estradiol levels in the pregnant women aged ≥ 40. The basal E_2 levels were not statistically significant between the conception and nonconception cycles.

Whether the aging human uterus has an age-related decrease in the basal uterine artery pulsatility index remains controversial, although Goswamy et al. (10) reported that uterine artery pulsatility index decreased with age and suggested a detrimental effect of age on uterine perfusion. In contrast, Cacciatore et al. (13) could not find any correlation between age and uterine artery pulsatility index or endometrial growth at the time of ET. Moreover, Guanes et al. (23) showed that age did not affect uterine resistance to vascular flow from week 6 of pregnancy to week 12 in pregnant patients receiving oocyte donation. Kurjak et al. (24) reported that the uterine artery resistance index did not change significantly in the first postmenopausal years, suggesting the thesis that the aging process initially affected the uterus less than the ovary.

Although several markers have been used to assess the ovarian reserve and pregnancy rate of IVF, no marker is available to optimally predict the pregnancy rate of IVF in women aged 40 and above. Basal FSH and AFC commonly have been used to predict the outcome of assisted reproduction (15,16,25). A previous report on basal FSH has shown that women with elevated basal serum FSH levels might have a lower pregnancy rate in IVF (15). The ongoing pregnancy rate per attempt of IVF were 18%, 15%, and 0% for women with basal FSH levels of ≤ 10 mIU/ml, 20 mIU/ml, and > 20 mIU/ml, respectively. The cancellation rate of IVF in women with basal FSH levels of < 15 mIU/ml, 20 mIU/ml, 25 mIU/ml, and ≥ 30 mIU/ml were 5%, 10%, 20%, and 40%, respectively (15). However, these criteria cannot predict the poor ovarian reserve in women who bear underlying abnormal ovarian function with a basal FSH level < 10, such as poor responders or those having undergone ovarian surgeries. Antral follicle count has been used to predict the ovarian response to hyperstimulation agents and pregnancy rate in an intrauterine insemination program (IUI) and assisted reproductive technologies (ART) (16,25). The antral follicle count significantly predicted the number of dominant follicle and retrieved oocytes. Moreover, patients with AFC < 5 in an IUI program and ≤ 3 in ART resulted in failed conception (16,25). However, Chang et al. (16) indicated that the AFC could predict only the oocyte number rather than the quality of the oocytes or the success of pregnancy in ART. In the ROC curve analysis of the present study, the use of basal UA PI was superior in predicting the pregnancy rate of IVF than using basal FSH or AFC. The best prediction rate was achieved by a PI cutoff of < 2.0 with a sensitivity of 75%, specificity of 84%, positive predict rate of 30%, and negative predictive rate of 97% for a receptive uterus.

In conclusion, basal uterine artery PI is more predictive of the pregnancy rate in women aged 40 and above as compared with day 3 FSH and AFC. Women aged ≥ 40 with lower basal UA PI have a better pregnancy rate, especially when the basal uterine artery PI is < 2.0. It therefore is essential that patients aged ≥ 40 with poor basal uterine perfusion should be identified earlier in the early follicular phase of the menstrual cycle to apply appropriate intervention to improve uterine circulation for the subsequent chance of pregnancy.

REFERENCES

1. Reuss ML, Kline J, Santos R, Levin B, Timor-Tritsh I: Age and the ovarian follicle pool assessed with transvaginal ultrasounography. Am J Obstet Gynecol 1996; 174:624–627
2. Bongain A, Castillon JM, Isnard V, Benoit B, Donzeau M, Gillet JY: In vitro fertilization in women over 40 years of age. A study on retrospective data for eight years. Eur J Obstet Gynecol Reprod Biol 1998;76:225–231
3. Lass A, Croucher C, Duffy S, Dawson K, Margara R, Winston RM: One thousand initiated cycles of in vitro fertilization in women > or = 40 years of age. Fertil Steril 1998;70:1030–1034
4. Dew JE, Don RA, Hughes GJ, Johnson TC, Steigrad SJ: The influence of advanced age on the outcome of assisted reproduction. J Assist Reprod Genet 1998;15:210–214
5. Romeu A, Muasher SJ, Acosta AA, Veeck LL, Diaz J, Jones GS, Jones HW Jr, Rosenwaks Z: Results of in vitro fertilization attempts in women 40 years of age and older: The Norfolk experience. Fertil Steril 1987;47:130–136
6. Toner JP, Flood JT: Fertility after the age of 40. Obstet Gynecol Clin North Am 1993;20:261–272
7. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF: Accelerated disappearance of ovarian follicles in mid-life: Implications for forecasting menopause. Hum Reprod 1992;7:1342–1346
8. Volarcik K, Sheean L, Goldfarb J, Woods L, Abdul-Karim FW, Hunt P: The meiotic competence of in-vitro matured human oocytes is influenced by donor age: evidence that folliculogenesis is compromised in the reproductively aged ovary. Hum Reprod 1998;13:154–160
9. Lim AS, Tsakok MF: Age-related decline in fertility: A link to degenerative oocytes? Fertil Steril 1997;68:265–271
10. Goswamy RK, Williams G, Steptoe PC: Decreased uterine perfusion—A cause of infertility. Hum Reprod 1988;3:955–959
11. Wada I, Hsu CC, Williams G, Macnamee MC, Brinsden PR: The benefits of low-dose aspirin therapy in women with impaired uterine perfusion during assisted conception. Hum Reprod 1994;9:1954–1957
12. Steer CV, Campbell S, Tan SL, Crayford T, Mills C, Mason BA, Collins WP: The use of transvaginal color flow imaging after in vitro fertilization to identify optimum uterine conditions before embryo transfer. Fertil Steril 1992;57:372–376
13. Cacciatore B, Simberg N, Fusaro P, Tiitinen A: Transvaginal Doppler study of uterine artery blood flow in in vitro fertilization–embryo transfer cycles. Fertil Steril 1996;66:130–134
14. Scott RT, Toner JP, Muasher SJ, Oehninger S, Robinson S, Rosenwaks Z: Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. Fertil Steril 1989;51:651–654
15. Toner JP, Philput CB, Jones GS, Muasher SJ: Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. Fertil Steril 1991;55:784–791
16. Chang MY, Chiang CH, Hsieh TT, Soong YK, Hsu KH: Use of the antral follicle count to predict the outcome of assisted reproductive technologies. Fert Steril 1998; 69:505–510
17. Marcus SF, Brinsden PR: In-vitro fertilization and embryo transfer in women aged 40 years and over. Hum Reprod Update 1996;2:459–468
18. Coulam CB, Bustillo M, Soenksen DM, Britten S: Ultrasonographic predictors of implantation after assisted reproduction. Fertil Steril 1994;62:1004–1010
19. Rubinstein M, Marazzi A, Polak de Fried E: Low-dose aspirin treatment improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation, and pregnancy rates in patients undergoing in vitro fertilization: A prospective, randomized, double-blind placebo-controlled assay. Fertil Steril 1999;71:825–829
20. de Ziegler D, Bessis R, Frydman R: Vascular resistance of uterine arteries: Physiological effects of estradiol and progesterone. Fertil Steril 1991;55:775–779
21. Hillard TC, Bourne TH, Whitehead MI, Crayford TB, Collins WP, Campbell S: Differential effects of transdermal estradiol

and sequential progestogens on impedance to flow within the uterine arteries of postmenopausal women. Fertil Steril 1992;58:959–963

22. Dickey RP, Hower JF: Relationship of estradiol and progesterone levels to uterine blood flow during early pregnancy. Early Pregnancy 1996;2:113–120
23. Guanes PP, Remohi J, Gallardo E, Valbuena D, Simon C, Pellicer A: Age does not affect uterine resistance to vascular flow in patients undergoing oocyte donation. Fertil Steril 1996;66:265–270
24. Kurjak A, Kupesic S: Ovarian senescence and its significance on uterine and ovarian perfusion. Fertil Steril 1995;64:532–537
25. Chang MY, Chiang CH, Chiu TH, Hsieh TT, Soong YK: The antral follicle count predicts the outcome of pregnancy in a controlled ovarian hyperstimulation/intrauterine insemination program. J Assist Reprod Genet 1998;15:12–17