Gynecological Endocrinology, July 2005; 21(1): 33–37

Taylor & Francis
Taylor & Francis Group

ASSISTED REPRODUCTIVE TECHNOLOGY

Antral follicle assessment after down-regulation may be a useful tool for predicting pregnancy loss in *in vitro* fertilization pregnancies

KORAY ELTER, ZEHRA N. KAVAK, HUSNU GOKASLAN, & TANJU PEKIN

Department of Obstetrics and Gynecology, Marmara University School of Medicine, Istanbul, Turkey

(Received 19 October 2004; revised 4 February 2005; accepted 7 February 2005)

Abstract
Women with diminished ovarian reserve (OR) have a high rate of pregnancy loss. The relationship between hormonal OR tests and pregnancy loss has been studied previously, but, to our knowledge, that between the antral follicle count (AFC) and pregnancy loss has not. Therefore, we aimed to determine whether OR tests, including the AFC, can predict pregnancy loss in women achieving pregnancy by means of *in vitro* fertilization (IVF), and also to compare their predictive value. All women underwent a fresh cycle of intracytoplasmic sperm injection with a long protocol with mid-luteal start of the gonadotropin-releasing hormone analog, and antral follicles were counted on cycle day 3 following down-regulation. Pregnancy losses up to 12 gestational weeks ($n = 28$) were compared with apparently healthy deliveries ($n = 34$) in this retrospective analysis. Receiver operating characteristic analysis of consecutive pregnancies ($n = 71$) was performed to analyze the optimum cut-off value for the significantly different OR tests. Women with a pregnancy loss had a lower AFC than those with healthy deliveries. Age and hormonal OR tests were comparable between groups. The optimum cut-off value for the AFC to predict pregnancy loss was 7.5. AFC may be a useful tool for predicting pregnancy loss in IVF pregnancies.

Keywords: *Antral follicle, biochemical pregnancy, ovarian reserve, pregnancy loss*

Introduction

Women with decreased ovarian reserve (OR) respond poorly to ovarian stimulation and have low pregnancy rates in *in vitro* fertilization (IVF) cycles, regardless of age. Sub-fertile women are commonly screened for decreased OR prior to IVF because of the poor IVF outcome in women with decreased OR. Among these screening tests, basal follicle-stimulating hormone (FSH) concentration and the antral follicle count (AFC) are more superior prognostically than age alone in predicting a woman's chance to conceive [1,2]. While dynamic tests, i.e., the clomiphene citrate challenge test, can be especially helpful as a screening test for decreased OR, the static tests, i.e., serum FSH level and the AFC, remain most practical for both patients and clinicians.

It has been suggested that women with diminished OR have a high rate of pregnancy loss [3–5]. This high loss rate is likely due to fetal aneuploidy. Recent publications have reported a relationship between decreased OR and Down's syndrome [6–8]. However, only hormonal OR tests have been undertaken in these studies. The AFC is more predictive of diminished OR than other static tests [2], and, to our knowledge, the relationship between this OR test and pregnancy loss has not been studied previously. Therefore, we aimed to determine whether OR tests, including the AFC, can predict early pregnancy losses up to 12 gestational weeks in IVF pregnancies, and also to compare their predictive value.

Methods

The present study is a retrospective analysis of 71 pregnancies which occurred following IVF–embyro transfer cycles between December 2001 and December 2003 in our assisted reproductive technologies center. Women with polycystic ovarian syndrome, habitual abortion (≥ 3 abortions), congenital or acquired uterine abnormality, who had a follicle of > 10 mm or evidence of an ovarian pathology on cycle day 3 of stimulation, were excluded from the study. All pregnant women underwent a fresh cycle of intracytoplasmic sperm injection (ICSI) with a long protocol with mid-luteal start of the gonadotropin-releasing hormone (GnRH) analog. Cycles were not necessarily the first cycles of subjects.

Correspondence: K. Elter, Kuyubasi S. Fenik Apt. No. 20/17, 34724 Feneryolu, Istanbul, Turkey. Tel: 90 532 265 2719. Fax: 90 216 428 0213.
E-mail: korayelter@marmara.edu.tr

ISSN 0951-3590 print/ISSN 1473-0766 online
DOI: 10.1080/09513590500099313

Women older than 45 years or who have a high (> 15 mIU/mL) basal serum FSH level are not offered IVF/ICSI treatment in our center. Institutional review board approval was obtained from the Marmara University School of Medicine.

In our center, treatment cycles were performed after a spontaneous cycle, during which a work-up, including hormonal OR tests and office hysteroscopy, was done. Hormonal OR tests, i.e., serum FSH and estradiol (E_2), were evaluated on day 3 of the spontaneous cycle preceding down-regulation. For the long protocol, pituitary desensitization was performed with subcutaneous daily injection of leuprolide acetate (Lucrin®; Abbott, Istanbul, Turkey) starting 1 week before the expected menses (mid-luteal start). Luteal administration of 1.0 mg/day was given during the luteal phase of the cycle preceding the stimulation cycle until the second day of the onset of menses, and decreased to 0.5 mg/day from day 2 until the day of injection of human chorionic gonadotropin (hCG).

After down-regulation was achieved (serum E_2 < 40 pg/ml), antral follicles were counted and ovarian stimulation with gonadotropins was commenced at cycle day 3 with a daily dose of 150–300 IU recombinant FSH (Gonal-F®; Serono, Istanbul, Turkey or Puregon®; Organon, Istanbul, Turkey) in combination with 300–600 IU of human menopausal gonadotropins (hMG) (Humegon®; Organon or Menogon®; Ferring, Istanbul, Turkey – both hMG contain 75 IU FSH and 75 IU luteinizing hormone). Starting dose was adjusted according to the patient's age, basal serum FSH and E_2 values at the preceding cycle and AFC. Round or oval echo-free structures were regarded as antral follicles, and all ovarian follicles measuring 2–10 mm on both ovaries were counted on day 3 of the stimulation cycle, i.e., after down-regulation. Antral follicle counts of both ovaries were added, and the total number per patient was used for calculations.

The subjects returned on day 6 or 7 of stimulation for an assessment of follicular recruitment and growth by transvaginal ultrasound. The gonadotropin dose and timing of subsequent scans were determined by the subject's response to controlled ovarian stimulation. When there were at least three follicles that were ⩾ 16 mm in diameter, hCG was administered. Transvaginal ultrasound was performed by three physicians, who were experienced in IVF, using a GE Logiq 200 Pro (GE Medical Systems, Milwaukee, WI, USA) with a 6.5-MHz vaginal transducer.

Transvaginal oocyte retrieval was performed 34–36 h later. All subjects received 10,000 IU of hCG. Approximately 4 h after the retrieval and just before sperm injection, oocytes were assessed for maturity, and metaphase II oocytes were injected. Approximately 18 h after injection, oocytes were checked for signs of fertilization.

Embryo transfer was performed on day 3 post-retrieval. Up to four transferable embryos were transferred. The luteal phase was supported by using progesterone in oil, 50 mg/day intramuscularly, starting on the day of ovum pick-up.

A quantitative serum hCG was determined 10 days after embryo transfer using an immunometric assay (Immulite®; Diagnostic Products Corporation, Los Angeles, CA, USA). The sensitivity of this assay is 1.1 mIU/mL, representing the lowest measurable hCG concentration that can be distinguished from zero. The intra-assay coefficient of variation is between 3.6 and 5.4%, with an inter-assay coefficient of variation between 7.8 and 9.9%. The pregnancy test was repeated 48 h after the initial test. A transvaginal ultrasound was performed when the hCG determination was > 1500 mIU/mL. A repeat ultrasound assessment was performed 1–2 weeks later to confirm fetal cardiac activity.

All pregnancies were followed for a minimum of 12 weeks of gestation and were subsequently classified as follows:

(1) A biochemical pregnancy loss was defined as β-hCG of > 10 mIU/mL that resolved spontaneously in the absence of ultrasound evidence of either an intrauterine or an ectopic pregnancy;
(2) The clinical spontaneous abortion group included all intrauterine pregnancies that were confirmed by ultrasound, but did not progress beyond the first trimester. These cases included clinical losses and pregnancies in which fetal cardiac activity could not be confirmed by the 12th week of gestation using transvaginal ultrasound;
(3) Ectopic pregnancies were diagnosed by laparoscopy;
(4) Pregnancies that were currently ongoing;
(5) Late abortions (between weeks 12 and 20 of gestation) and stillbirths; and
(6) Apparently healthy deliveries – these apparently healthy babies had no minor or major anomaly.

All biochemical and clinical pregnancy losses (Groups 1 and 2 in combination) were compared with apparently healthy deliveries (Group 6) in this retrospective analysis. Subsequently, biochemical pregnancy losses (Group 1) alone were compared with apparently healthy deliveries (Group 6). Statistical analysis involved univariate comparisons between these groups by using Student's t, the Mann–Whitney U, χ^2 and Fisher's exact tests, where appropriate. Parameters in Table I were compared between groups.

Receiver operating characteristic (ROC) analysis was performed to analyze the predictive value of OR tests for the pregnancy loss. All pregnancies ($n = 71$) were included in this analysis. Diagnostic sensitivity and specificity were calculated, and the ROC curve

Table I. Characteristics of women with an abortion and those with a healthy delivery following a pregnancy achieved by *in vitro* fertilization (IVF).

	All abortions (< 12 gestational weeks) ($n = 28$)	Deliveries ($n = 34$)	p Value
Age (years)	30.0 ± 5.3 (27.9–32.0)	30.8 ± 5.0 (29.1–32.6)	0.51
Body mass index (kg/m^2)	24.0 ± 3.7 (22.4–25.5)	22.8 ± 3.1 (21.7–23.9)	0.21
Duration of infertility (years)	4.3 ± 3.1 (3.3–6.0)	5.8 ± 4.2 (4.3–7.4)	0.11
Rate of women with primary infertility (%)	82	71	0.29
Rate of women with previous abortions (%)	14	18	0.72
Rate of women with previous IVF failures (%)	36	44	0.50
Basal serum FSH level (mIU/ml)	6.7 ± 2.8 (5.6–7.8)	6.5 ± 1.4 (5.9–7.1)	0.76
Basal serum E_2 level (pg/ml)	45.9 ± 31.0 (33.6–58.1)	34.6 ± 17.0 (27.8–41.5)	0.11
Antral follicle count	6.8 ± 2.4 (5.9–7.8)	8.8 ± 3.6 (7.5–10.1)	0.02
Number of oocytes retrieved	10.5 ± 4.3 (8.9–12.2)	13.8 ± 6.2 (11.6–16.0)	0.02
Number of mature (metaphase II) oocytes retrieved	8.3 ± 3.6 (6.8–9.7)	10.9 ± 4.9 (9.2–12.6)	0.02
Number of embryos transferred	2.9 ± 0.6 (2.6–3.1)	3.1 ± 0.6 (2.8–3.3)	0.26
Number of appropriately cleaving embryos (6–8-cell stage) transferred	2.5 ± 1.0 (2.2–2.9)	2.9 ± 0.8 (2.6–3.2)	0.13
Number of grade 1 embryos transferred	2.0 ± 1.3 (1.5–2.5)	2.4 ± 1.2 (1.9–2.8)	0.22

FSH, follicle-stimulating hormone; E_2, estradiol; values are expressed as mean ± standard deviation (95% confidence interval).

was constructed by plotting the sensitivity against the false-positive rate (1–specificity) of various cut-off values for predicting pregnancy loss. The value with the optimal combination of sensitivity and specificity was chosen as the cut-off value. Area under each ROC curve (AUC_{ROC}), which indicates the predictive power of the parameter, was calculated. The p value of the ROC analysis indicates the significance of the difference between the relevant variable and the coin test, which has an AUC of 0.5. SPSS release 11.5 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Values are expressed as mean ± standard deviation, and a p value of < 0.05 was considered significant.

Results

Nineteen women had biochemical pregnancy loss, nine had a clinical spontaneous abortion of < 12 gestational weeks, one had a late abortion, one had a stillbirth, one had an ectopic pregnancy, six had an ongoing pregnancy and 34 women had apparently healthy deliveries.

When all abortions up to 12 gestational weeks, including biochemical pregnancy losses, were compared with healthy deliveries, demographic characteristics were comparable (Table I). However, OR, as determined by the AFC, and the number of oocytes retrieved were lower in women with abortions (Table I). Hormonal OR tests and quantity and quality of embryos transferred were comparable between groups (Table I). Similar results were obtained when clinical spontaneous abortions were excluded, and only biochemical pregnancy losses were compared with healthy deliveries (Table II).

The predictive roles of age and OR tests, as expressed by AUC_{ROC}, are shown in Table III. For predicting both all abortions that were < 12 gestational weeks including biochemical pregnancy losses and the biochemical pregnancy losses alone, only the AFC had significant value (Table III). The optimum cut-off value was 7.5 (Table III). The odds ratio (95% confidence interval) was 4.2 (1.5–11.8) for abortion, and 5.5 (1.6–19.0) for biochemical pregnancy loss. Women with an AFC of < 7.5 on day 3 of stimulation were 4.2 times more likely to have an abortion and 5.5 times more likely to have a biochemical pregnancy loss following an IVF pregnancy, compared with those who had more than 7.5 antral follicles.

Discussion

The aim of the present study was to compare the OR of patients with a pregnancy loss with that of patients having an apparently healthy delivery. The vast majority of losses were early losses, i.e., prior to the detection of a clinical pregnancy. We subsequently analyzed an optimum cut-off value for the only significant OR test, i.e., the AFC, in the prediction of pregnancy loss after a documented biochemical pregnancy. The present study demonstrates that pregnancy loss prior to the 12th week of gestation in IVF pregnancies is associated with decreased OR as determined by the AFC. Pregnant women who had an AFC < 7.5 and got pregnant following a long IVF protocol were approximately 4 to 6 times more likely to have an early or clinical pregnancy loss than were pregnant women who had more antral follicles.

It is known that the risk of spontaneous abortion increases with increasing maternal age [9]. Age is also a marker of OR in women with sub-fertility. It has been reported that patients with unexplained recurrent pregnancy loss have higher basal FSH levels than do patients with explained recurrent pregnancy loss [5]. There is also a relationship between diminished OR and Down's syndrome pregnancies [6]. It has been reported that women

Table II. Characteristics of women with a biochemical pregnancy loss and those with a healthy delivery following a pregnancy achieved by *in vitro* fertilization (IVF).

	Biochemical pregnancy losses ($n = 19$)	Deliveries ($n = 34$)	p Value
Age (years)	29.7 ± 5.7 (27.0–32.5)	30.8 ± 5.0 (29.1–32.6)	0.27
Body mass index (kg/m^2)	24.0 ± 4.1 (21.9–26.2)	22.8 ± 3.1 (21.7–23.9)	0.10
Duration of infertility (years)	3.8 ± 2.3 (2.6–4.9)	5.8 ± 4.2 (4.3–7.4)	0.23
Rate of women with primary infertility (%)	84	71	0.27
Rate of women with previous abortions (%)	11	18	0.70
Rate of women with previous IVF failures (%)	32	44	0.37
Basal serum FSH level (mIU/ml)	6.6 ± 2.8 (5.2–8.0)	6.5 ± 1.4 (5.9–7.1)	0.49
Basal serum E_2 level (pg/ml)	41.2 ± 28.3 (27.6–54.9)	34.6 ± 17.0 (27.8–41.5)	0.49
Antral follicle count	6.4 ± 2.0 (5.4–7.4)	8.8 ± 3.6 (7.5–10.1)	0.006
Number of oocytes retrieved	10.7 ± 4.6 (8.5–13.0)	13.8 ± 6.2 (11.6–16.0)	0.03
Number of mature (metaphase II) oocytes retrieved	8.8 ± 3.9 (6.9–10.6)	10.9 ± 4.9 (9.2–12.6)	0.04
Number of embryos transferred	2.8 ± 0.6 (2.6–3.1)	3.1 ± 0.6 (2.8–3.3)	0.21
Number of appropriately cleaving embryos (6–8-cell stage) transferred	2.4 ± 1.0 (2.0–2.9)	2.9 ± 0.8 (2.6–3.2)	0.07
Number of grade 1 embryos transferred	2.1 ± 1.3 (1.4–2.7)	2.4 ± 1.2 (1.9–2.8)	0.37

FSH, follicle-stimulating hormone; E_2, estradiol; values are expressed as mean ± standard deviation (95% confidence interval).

Table III. Results of the receiver operating characteristic analysis. Optimum cut-off values for significantly different parameters and predictive values for these parameters.

	AUC ± SE	p Value*	95% CI	Cut-off	Sn (%)	Sp (%)	PPV (%)	NPV (%)
Predictive value for all abortions								
Age (years)	0.43 ± 0.07	0.29	0.29–0.57	NA	NA	NA	NA	NA
Basal serum FSH level (mIU/ml)	0.56 ± 0.08	0.42	0.41–0.71	NA	NA	NA	NA	NA
Basal serum E_2 level (pg/ml)	0.57 ± 0.07	0.32	0.43–0.72	NA	NA	NA	NA	NA
Antral follicle count	0.65 ± 0.07	0.04	0.52–0.78	7.5	71	63	56	77
Predictive value for biochemical pregnancy loss								
Age (years)	0.41 ± 0.08	0.27	0.26–0.57	NA	NA	NA	NA	NA
Basal serum FSH level (mIU/ml)	0.53 ± 0.09	0.70	0.37–0.70	NA	NA	NA	NA	NA
Basal serum E_2 level (pg/ml)	0.49 ± 0.08	0.95	0.34–0.65	NA	NA	NA	NA	NA
Antral follicle count	0.68 ± 0.07	0.02	0.55–0.81	7.5	79	60	42	89

AUC, area under the curve; SE, standard error; CI, confidence interval; Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; FSH, follicle-stimulating hormone; E_2, estradiol; NA, not applicable; *significance of the difference from a coin test, which has an AUC of 0.5.

with a history of a Down's syndrome pregnancy have significantly elevated basal FSH concentration [7]. Moreover, women having a recent abortion of a conception with an abnormal karyotype were found to have elevated basal FSH and E_2 concentrations [10]. Another study reported that women with a reduced ovarian tissue (either by surgical removal or congenital absence of one ovary) were more likely to have delivered a child with Down's syndrome [8]. Therefore, it appears to be more than a hypothesis that women with diminished OR have significantly higher rates of pregnancy loss than do patients with normal OR. Indeed, Levi and colleagues reported that 71% of 28 pregnancies of women with diminished OR (FSH $\geqslant$ 14.2 IU/l) were lost in the first trimester [3]. The rate of pregnancy loss in these women was significantly higher than in age-matched women with normal OR [3].

However, to our knowledge, the AFC has not been studied previously as a predictive marker of abortion, although it has been demonstrated that the AFC provides better prognostic information on the occurrence of poor response during ovarian stimulation for IVF than do the patient's chronological age and the currently used endocrine markers [2]. The AFC has also been incorporated in many assisted reproduction programmes owing to its ease of use, because of the prerequisite for a basal ultrasound examination in IVF.

While the abortuses were not routinely karyotyped in this study, the high loss rate in patients with a low AFC was likely due to fetal aneuploidy, although this cannot be known for certain. Poor-prognosis patients in IVF have high embryonic aneuploidy rates despite morphologically and developmentally normal appearing embryos [11,12]. The relationship between OR and Down's syndrome suggests that preconceptional screening for decreased OR could become useful in identifying women at risk for aneuploid pregnancies.

Antral follicles were counted after pituitary down-regulation in the present study. Although this may seem a limitation of the study, it has been shown that pituitary down-regulation with a GnRH agonist does not result in a significant change in the AFC, and ultrasound examinations for the AFC can be reliably performed after pituitary down-regulation [13–15]. In our center, three experienced physicians perform all scans. This also appears to be a weakness of the study. However, it has been shown that inter-observer variability for the AFC is acceptable, even for more than two examiners [14,16].

Age was not a confounding variable in the present study, since it was comparable between groups. The fact that age and hormonal OR tests were comparable, and not found to be predictors for pregnancy loss in the present study, may be due to a type II error. It should be mentioned that the limited number of subjects is a weakness of the present study. However, the present study clearly showed that the AFC following down-regulation is better than age and hormonal OR tests, i.e., serum FSH and E_2 levels, in predicting pregnancy loss. This result is consistent with the fact that the AFC provides better prognostic information on OR than do the patient's chronological age and the currently used endocrine markers [2].

The results in the present study might assist physicians and their patients with the decision-making process prior to embarking upon fertility therapy. Our results showed that when patients with diminished OR do conceive, they have a high rate of reproductive loss, regardless of age.

In conclusion, women with a low AFC following down-regulation have a high risk of miscarriage after an IVF pregnancy. These women should be counseled about the high rate of reproductive loss associated with diminished OR, in addition to their poor prognosis of conception in general.

References

1. 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;55:784–91.
2. Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in *in vitro* fertilization: a prospective study comparing basal markers of ovarian reserve. Fertil Steril 2002;77:328–36.
3. Levi AJ, Raynault MF, Bergh PA, Drews MR, Miller BT, Scott RT Jr. Reproductive outcome in patients with diminished ovarian reserve. Fertil Steril 2001;76:666–9.
4. Hofmann GE, Khoury J, Thie J. Recurrent pregnancy loss and diminished ovarian reserve. Fertil Steril 2000;74:1192–5.
5. Trout SW, Seifer DB. Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values? Fertil Steril 2000;74:335–7.
6. van Montfrans JM, Lambalk CB, van Hooff MH, van Vugt JM. Are elevated FSH concentrations in the pre-conceptional period a risk factor for Down's syndrome pregnancies? Hum Reprod 2001;16:1270–3.
7. van Montfrans JM, Dorland M, Oosterhuis GJ, van Vugt JM, Rekers-Mombarg LT, Lambalk CB. Increased concentrations of follicle-stimulating hormone in mothers of children with Down's syndrome. Lancet 1999;353:1853–4.
8. Freeman SB, Yang Q, Allran K, Taft LF, Sherman SL. Women with a reduced ovarian complement may have an increased risk for a child with Down syndrome. Am J Hum Genet 2000;66:1680–3.
9. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. Br Med J 2000;320:1708–12.
10. Nasseri A, Mukherjee T, Grifo JA, Noyes N, Krey L, Copperman AB. Elevated day 3 serum follicle stimulating hormone and/or estradiol may predict fetal aneuploidy. Fertil Steril 1999;71:715–18.
11. Munne S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. Fertil Steril 1995;64:382–91.
12. Magli MC, Gianaroli L, Munne S, Ferraretti AP. Incidence of chromosomal abnormalities from a morphologically normal cohort of embryos in poor-prognosis patients. J Assist Reprod Genet 1998;15:297–301.
13. Sharara FI, Lim J, McClamrock HD. The effect of pituitary desensitization on ovarian volume measurements prior to *in-vitro* fertilization. Hum Reprod 1999;14:183–5.
14. Hansen KR, Morris JL, Thyer AC, Soules MR. Reproductive aging and variability in the ovarian antral follicle count: application in the clinical setting. Fertil Steril 2003;80:577–83.
15. Yong PY, Baird DT, Thong KJ, McNeilly AS, Anderson RA. Prospective analysis of the relationships between the ovarian follicle cohort and basal FSH concentration, the inhibin response to exogenous FSH and ovarian follicle number at different stages of the normal menstrual cycle and after pituitary down-regulation. Hum Reprod 2003;18:35–44.
16. Scheffer GJ, Broekmans FJ, Bancsi LF, Habbema JD, Looman CW, te Velde ER. Quantitative transvaginal two- and three-dimensional sonography of the ovaries: reproducibility of antral follicle counts. Ultrasound Obstet Gynecol 2002;20:270–5.