Diminished ovarian reserve: is it a neglected cause in the assessment of recurrent miscarriage? A cohort study

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Objective: To study whether diminished ovarian reserve is associated with recurrent miscarriage.
Design: Cross-sectional clinical study.
Setting: Tertiary-care center.
Patient(s): Women with history of recurrent miscarriage (RM; n = 71) and sequentially selected age-matched fertile women who were seeking contraception (control; n = 70).
Intervention(s): Not applicable.
Main Outcome Measures(s): Serum levels of FSH, LH, E_2, and antimüllerian hormone (AMH); FSH/LH ratio; ovarian volumes; and antral follicle count (AFC).
Result(s): The levels of FSH were 8.6 ± 3.7 U/L in the RM group and 7.1 ± 3.9 U/L in the control group; this difference was statistically significant. The levels of AMH were significantly lower in the RM group than in the control group (2.9 ± 1.7 ng/mL vs. 3.6 ± 1.7 ng/mL). The percentage of women with levels of FSH ≥ 11 U/L was significantly higher in the RM group than in the control group (18.3% vs. 4.3%). In the RM group, the percentage of women with levels of AMH ≤ 1 ng/mL was significantly higher than in the control group (19.7% vs. 5.7%).
Conclusion(s): Recurrent miscarriage may be associated with diminished ovarian reserve. Larger prospective randomized controlled trials are warranted to better determine the predictive potential of ovarian reserve markers in recurrent miscarriage. (Fertil Steril® 2016;105:1236–40. ©2016 by American Society for Reproductive Medicine.)
Key Words: Antimüllerian hormone, antral follicle count, diminished ovarian reserve, ovarian reserve markers, recurrent miscarriage

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Recurrent miscarriage (RM) is defined as three or more failed clinical pregnancies at <20 weeks of gestation or fetal weight <500 g. The estimated incidence of RM is reported as between 1% and 5% of woman of reproductive age (1). Known causes of RM include antiphospholipid antibodies, uterine anomalies, endocrine disorders, infectious diseases, immune factors, thrombophilias, and parental abnormal chromosomes (2–5). Approximately 50% of cases of RM do not have a clearly defined etiology and are classified as unexplained (6, 7). This high percentage suggests that current evaluation methods for women with RM are insufficient and that different etiologic factors should be investigated.

Ovarian reserve demonstrates reproductive potential as the number and quality of remaining oocytes (8, 9). Ovarian reserve tests include measurements of FSH, E_2, inhibin B, and antimüllerian hormone (AMH) levels. Sonographic assessment of antral follicle count (AFC) and ovarian volume also reflect ovarian reserve (10). An elevated basal FSH level is used clinically as a marker for diminished ovarian reserve (DOR) (11, 12). Basal serum FSH concentrations increase on day 2, 3, or 4 of the

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menstrual cycle with advancing reproductive age. In this regard, biologic age is more important than chronologic age, because there is an age-independent relationship between elevated basal FSH level and reduced oocyte quality/aneuploidy risk (13). AMH is a novel marker of ovarian reserve and a good predictor of oocyte quantity. Levels of AMH are stable within and between menstrual cycles. Decreased AMH levels are associated with poor ovarian response to stimulation (10).

The association between advanced maternal age and RM indicates that DOR may have a possible connection with future pregnancy prognosis. The purpose of the present study was to investigate whether DOR is associated with RM.

MATERIALS AND METHODS

This study was conducted at the obstetrics and gynecology department of a tertiary-care center from 2011 to 2015. After the approval of the local Institutional Review Board (2011/14A) was obtained, and informed consents of all subjects were received, the study was performed. RM was defined as three or more pregnancy losses at <20 weeks of gestation or fetal weight <500 g. The 71 women with history of RM for whom routine workup for RM (chromosomal analyses of both partners; levels of prolactin and TSH; anticardiolipin antibody, lupus anticoagulant, antinuclear antibody, and coagulation studies; and pelvic ultrasonography) was negative were assigned to the RM group. The control group consisted of sequentially selected 70 healthy women with no history of RM who were seeking contraception in the center's family planning unit.

The exclusion criteria were diagnosis of polycystic ovarian syndrome or anovulation; the presence of endometriosis as indicated by laparoscopic or ultrasonographic evidence; a history of ovarian surgery, tobacco use, systemic chemotherapy, pelvic irradiation, genetic abnormalities, or irregular menstrual cycles; a familial history of premature ovarian failure; the existence of ovarian follicles >10 mm in diameter during the early follicular phase; and the use of oral contraceptives or other hormone therapy within the preceding 3 months.

Venous blood samples were taken from the antecubital regions of all patients between 8:00 a.m. and 9:00 a.m. during the early follicular phase (days 2–4) of the menstrual cycle. Serum samples were stored at −80°C and assayed for FSH, LH, E_2, and AMH. FSH levels were analyzed by means of an electrochemiluminescence method that involved use of the Advia Centaur XP Immunoassay System (Siemens Healthcare Diagnostics). The normal range for this assay is 2.5–10 U/L at the early follicular phase. The coefficient of variation (CV) is 6%. Serum AMH levels were measured with the use of a human ELISA kit according to the manufacturer's instructions (YH Biosearch). The normal range for this assay is 0.05–1.5 ng/mL. The coefficients of intra- and interassay variations are <10% and <12%, respectively. In the same morning that the blood tests were performed, ovarian volume and the total numbers of antral follicles measuring 2–10 mm in diameter were evaluated by the same operator, who was blinded to patient information. A 7.5-MHz transvaginal probe (SonoAce X8 Ultrasound; Samsung Medison) was used in all examinations. Ovarian volume was calculated by means of the equation for ellipsoid volume (length × width × thickness × 0.523).

Demographic data (including age, gravidity, parity, pregnancy loss, and body mass index) and ovarian reserve parameters (including serum levels of AMH, FSH, LH, and E_2; FSH/LH ratios; right and left ovarian volumes; and AFCs for both ovaries) were noted for both groups, and the two groups were compared regarding all of these factors. The cutoff values of poor ovarian reserve markers were defined as a serum FSH level ≥11 U/L, a serum E_2 level ≥ 60 nmol/L, an FSH/LH ratio of ≥3, an AMH level of ≤1 ng/mL, and a total AFC (TAFC) of ≤7 (10).

Data were analyzed with the use of IBM's SPSS software (SPSS version 15.0 for Windows); $P<.05$ was considered to be statistically significant. Mean, median, SD, lowest and highest frequency, and ratio values are used at statistical complementary of data. Quantitative data were analyzed with the use of the Student *t* test and the Mann-Whitney *U* test. A chi-square test was used for analyses of qualitative data.

RESULTS

The RM group consisted of 71 women who had had three or more pregnancy losses and met the eligibility criteria for the study. The control group consisted of 70 fertile women with no history of recurrent miscarriage who were seeking contraception. The descriptive data and variables indicating ovarian reserve are presented in Table 1. There was no statistically significant difference between the groups regarding mean menstrual cycle length or body mass index. There were statistically significant differences in gravidity, parity, and pregnancy loss between the RM group and the control group. Mean age (29.5 ± 4.5 y vs. 29.1 ± 4.7 y) and the percentage of women within the ages of ≤30 years and >30 years (59.2% vs. 61.4% and 40.8% vs. 38.6%, respectively) were similar in the RM and control groups (Table 1).

The levels of FSH were 8.6 ± 3.7 U/L in the RM group and 7.1 ± 3.9 U/L in the control group; this difference was statistically significant ($P=.049$). In the RM group, 13 of the 71 women (18.3%) had levels of FSH ≥11 U/L, whereas only three of the 70 women (4.3%) in the control group did ($P=.009$; Table 1; Fig. 1).

The levels of AMH were 2.9 ± 1.7 ng/mL in the RM group and 3.6 ± 1.7 ng/mL in the control group ($P=.007$). The percentage of women with levels of AMH ≤1 ng/mL was 19.7% in the RM group and 5.7% in the control group ($P=.013$; Table 1; Fig. 1).

The levels of LH, FSH/LH ratios, and E_2 were similar between the two groups. The percentage of women with FSH/LH ≥3 and E_2 ≥60 nmol/L did not differ significantly between the two groups (Table 1).

The RM and control groups were divided into two subgroups based on age (≤30 y and >30 y). The percentage of women with levels of FSH ≥11 U/L did not differ significantly between the RM and control groups in both age subgroups (Table 2). The percentage of women with levels of AMH ≤1 ng/mL was similar in the RM and control groups

TABLE 1

Comparison of demographic characteristics and ovarian reserve test parameters between recurrent miscarriage and control groups.

Parameter	Recurrent miscarriage (n = 71)	Control (n = 70)	*P* value
Age (y)	29.5 ± 4.5	29.1 ± 4.7	NS
≤30	42 (59.2%)	43 (61.4%)	NS
>30	29 (40.8%)	27 (38.6%)	
BMI (kg/m^2)	24 ± 3.2	25 ± 3.9	NS
Mean cycle length (d)	28.3 ± 2.2	28.5 ± 1.5	NS
Gravidity	3.7 ± 0.9	1.7 ± 0.6	.001
Parity	0.2 ± 0.4	1.5 ± 0.7	.001
Pregnancy loss	3.5 ± 0.9	0.09 ± 0.2	.001
FSH (U/L)	8.6 ± 3.7	7.1 ± 1.9	.049
FSH ≥11 U/L	13 (18.3%)	3 (4.3%)	.009
LH (U/L)	5.2 ± 2.2	5.1 ± 2.4	NS
E_2 (nmol/L)	42.2 ± 15.1	45.5 ± 30.2	NS
E_2 ≥60 nmol/L	7 (9.9%)	12 (17.1%)	NS
FSH/LH	1.7 ± 0.7	1.6 ± 1.1	NS
FSH/LH ≥3	4 (5.6%)	5 (7.1%)	NS
ROV (mL)	6.0 ± 2.3	6.1 ± 1.7	NS
LOV (mL)	6.1 ± 2.2	6.0 ± 1.7	NS
MOV (mL)	6.0 ± 2.0	6.1 ± 1.6	NS
ROAFC (n)	4.9 ± 2.0	5.0 ± 2.0	NS
LOAFC (n)	5.1 ± 2.2	4.7 ± 2.0	NS
TAFC (n)	9.4 ± 4.0	9.8 ± 3.8	NS
TAFC ≤7	27 (38%)	19 (27.1%)	NS
AMH (ng/mL)	2.9 ± 1.7	3.6 ± 1.7	.007
AMH ≤1 ng/mL	14 (19.7%)	4 (5.7%)	.013

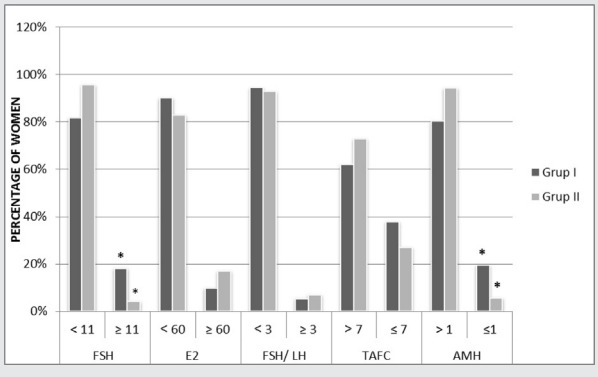
Note: Results are presented as mean ± SD or n (%). AMH = antimüllerian hormone; BMI = body mass index; LOAFC = left ovary antral follicle count; LOV = left ovarian volume; MOV = mean ovarian volume; NS = not significant; ROAFC = right ovary antral follicle count; ROV = right ovarian volume; TAFC = total antral follicle count.

Atasever. Ovarian reserve in recurrent miscarriage. Fertil Steril 2016.

in the age ≤30 years subgroup (P>.05; Table 2). The percentage of women with levels of AMH ≤1 ng/mL was 34.5% in the RM group and 7.4% in the control group in the age >30 years subgroup (P=.021; Table 2). However, the percentages of women with levels of E_2 ≥60 nmol/L and FSH/LH ≥3 were not significantly different between the RM and control groups in both age subgroups (Table 2).

The right ovarian volume (ROV), left ovarian volume (LOV), mean ovarian volume (MOV), right ovary AFC (ROAFC), left ovary AFC (LOAFC), and TAFC were not statistically significantly different between the RM and control groups, as presented in Table 1. The percentage of women with TAFC ≤7 was not statistically different between the two groups or between the age subgroups (Table 1; Fig. 1).

FIGURE 1



Percentage of women with diminished ovarian reserve in the recurrent miscarriage and control groups. TAFC = total antral follicle count; AMH = antimüllerian hormone.

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DISCUSSION

This cross-sectional study showed that RM may be associated with DOR as measured by serum FSH and AMH levels. The data indicate that women with RM had DOR, regardless of maternal age. To the best of the authors' knowledge, this is the first large prospective cohort study investigating the role of multiple ovarian reserve markers such as FSH, LH, E_2, AMH, TAFC, FSH/LH ratio, and ovarian volume all together in RM. AMH, AFC, and ovarian volume had not to date been assessed as a marker of DOR in women with RM.

Chromosomal abnormalities are observed in embryos in connection with decreased quality of the oocyte, which has been reported as a reason of miscarriage in 35%–75% of all cases (14, 15). Various studies have suggested that there is a relationship between DOR and chromosomal abnormalities in the products of conception (16, 17). Conversely, some studies seem to have demonstrated the absence of an association between quantitative ovarian reserve and miscarriage or chromosomal abnormalities (18–20). Most of these studies have a small sample size and retrospective design. However, a study among of subfertile women has suggested that basal FSH levels, clomiphene citrate challenge test results, and AFC are not predictive for the chance of miscarriage (21).

TABLE 2

Comparison of the ovarian reserve tests according to age groups.

	Age ≤30 y			Age >30 y		
Parameter	**RM**	**Control**	***P* value**	**RM**	**Control**	***P* value**
FSH ≥11 U/L	5 (11.9)	1 (2.3)	NS	8 (27.6)	2 (7.4)	NS
E_2 ≥60 nmol/L	4 (9.5)	6 (14)	NS	3 (10.3)	6 (22.2)	NS
FSH/LH ≥3	1 (2.4)	2 (4.7)	NS	3 (10.3)	3 (11.1)	NS
TAFC ≤7	9 (21.4)	5 (11.6)	NS	18 (62.1)	14 (51.9)	NS
AMH ≤1 ng/mL	4 (9.5)	2 (4.7)	NS	10 (34.5)	2 (7.4)	.021

Note: Results are presented as n (%). RM = recurrent miscarriage group; other abbreviations as in Table 1.

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A retrospective cohort study found that aneuploidy is the most common cause of pregnancy losses in patients with recurrent (≥3) miscarriage over the age of 35 years (22). In another study, Hofmann et al. demonstrated that women with unexplained recurrent pregnancy loss (RPL) have an incidence of DOR that is similar to that of the general infertile population (23). Numerous studies have identified an increased rate of chromosomal abnormalities in embryos derived from couples with RM (24–26). Therefore, it is possible that aneuploidy due to DOR is an additional contributing cause for RM. The present study has attempted to determine whether the parameters related to ovarian reserve are altered in patients with RM.

In this study, the percentage of women with levels of FSH ≥11 U/L was found to be statistically significantly higher in the recurrent miscarriage group than in the control group. Similarly, Trout et al. reported that women with unexplained RPL have a higher incidence of elevated day 3 serum FSH and E_2 levels than do women with a known cause of RPL (27). A retrospective comparative analysis found that day 3 E_2 levels and FSH/LH ratios were higher in women with unexplained RPL than in control women (28).

It has been demonstrated that AFC predicts poor response much better than does basal FSH (29). In the present study, there was no difference between groups regarding TAFC. However, this examination is an operator- and machine-dependent procedure. The results may have been affected by difficulties in obtaining a correct AFC, such as intra-observer variability and anatomic variations.

Use of serum AMH level is a recently introduced method for assessment of ovarian reserve. AMH and AFC are more reliable markers of ovarian reserve than FSH, because basal FSH levels can vary from cycle to cycle. Several studies have found that there is a significant positive correlation between AMH levels and the quality (30) and quantity (31) of oocytes, although the value of AMH in predicting oocyte quality is controversial (32). In the present study, the percentage of women with levels of AMH ≤1 ng/mL was statistically significantly higher in the recurrent miscarriage group than in the control group. This may suggest a causal relationship between RM and DOR; further study is warranted.

DOR and advanced biologic ovarian aging are better predictors of aneuploidy risk than is chronologic age. Similarly, it has been reported that unexplained infertility has a connection with DOR regardless of age (11). The present study found that the percentage of women with levels of FSH ≥11 was not different in the RM group than in the control group or between both age subgroups (women of ages ≤30 y and >30 y). The present study found that in the age >30 y subgroups, the percentage of women with levels of AMH ≤1 ng/mL was significantly higher in the RM group than in the control group. According to these results, DOR may be a predictive value for RM.

One limitation of the present study may be the absence of cytogenetic testing of the miscarriages. However, the study has several strengths compared with earlier reports. These strengths include a large sample size, the study of multiple markers, prospective design, and relatively homogeneous groups of subjects.

CONCLUSION

The present study found that the percentage of women with levels of FSH ≥11 U/L and AMH ≤1 ng/mL was statistically significantly higher in the RM group than in the control group. This finding suggests that there may be a relationship between elevated FSH, decreased AMH (both of which are markers of ovarian reserve), and RM. Larger prospective randomized controlled trials are warranted to better determine the predictive potential of ovarian reserve markers in recurrent miscarriage.

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