ORIGINAL ARTICLE: REPRODUCTIVE ENDOCRINOLOGY

Ethnicity as a determinant of ovarian reserve: differences in ovarian aging between Spanish and Indian women

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Objective: To investigate differences in ovarian reserve markers (antimüllerian hormone [AMH] and antral follicle count [AFC]) in Indian and Spanish women.
Design: Cross-sectional study.
Setting: In vitro fertilization (IVF) clinics.
Patient(s): Infertile Spanish (n = 229) and Indian (n = 236) women who underwent controlled ovarian stimulation for IVF from January to October 2012.
Intervention(s): None.
Main Outcome Measure(s): Data on ovarian reserve markers and results after ovarian stimulation were collected.
Result(s): The mean age of women undergoing their first or second IVF cycle was significantly higher in Spanish than in Indian women (37.5 ± 3.3 years vs. 31.5 ± 3.8 years). Despite this 6-year age gap, AFCs were similar (9.5 ± 4.7 vs. 9.9 ± 4.6), as were day 3 FSH levels (7.5 ± 4.5 IU/L vs. 6.9 ± 2.3 IU/L). AMH levels were slightly lower in Spanish women (1.6 ± 1.7 ng/mL vs. 2.5 ± 1.6 ng/mL). Multivariate regression analysis showed that being Indian decreased AFC by 2.3, such that AFC in Indian women was similar to that in Spanish women 6.3 years older (95% confidence interval 3.39–1.10).
Conclusion(s): Similar ovarian reserve markers and ovarian response were observed in women with a 6-year age difference in favor of the Spanish, suggesting ethnic differences in ovarian aging. Further research is needed to understand whether these differences are genetically induced or are caused by other variables, such as nutrition. Our results may help clinicians to counsel infertile women when discussing assisted reproductive technology outcomes according to age and ethnic background. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Ethnicity, ovarian aging, AMH, AFC

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There is growing evidence that female reproductive function may differ by race. Ethnicity strongly influences the prevalence of several gynecologic diseases. For example, patients of South Asian Indian descent have higher rates of insulin resistance and polycystic ovary syndrome (1). Puberty onset has been reported to start earlier in Asian American girls than in white American girls (2). Considering the increasing demand for assisted reproductive technology (ART) as well as the strong influence of ovarian reserve on ART outcome, it might be found that ovarian reserve may also differ according to ethnic origins. This difference may have significant implications when evaluating ovarian reserve to categorize patients and to establish prognoses for ovarian response, thus avoiding excessive response or prescribing adequate protocols for suspected poor responders.

Ethnicity has been consistently shown to affect ART outcome (3–8). Some ethnic groups (Asian, African American, and Hispanic) have significantly lower clinical pregnancy and live birth rates and higher miscarriage rates after ART than whites (6). Despite younger age and similar embryo quality, Indian American women had a significantly lower live birth rate than white American women in an earlier investigation (9).

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Earlier studies have focused on basal hormonal fluctuations in various ethnic populations. Day 3 FSH levels were higher in African American women than in age-matched white women (10–12). Similarly, antimüllerian hormone (AMH) levels were lower in African-American and Hispanic women (25.2% and 24.6% lower, respectively) than in white women (13). Thus, there may be an independent effect of race and ethnicity on the age-related declines in AMH levels and ovarian function over time.

The purpose of the present study was to investigate whether there were any differences in ovarian reserve markers (AMH and antral follicle count [AFC]) between age-matched populations of Indian and Spanish women.

MATERIALS AND METHODS

Experimental Design

We conducted a prospective cohort study of 465 infertile Spanish and Indian women who underwent controlled ovarian stimulation (COS) for in vitro fertilization (IVF) from January to October 2012 in IVF centers in Spain and India. The population included prospectively in this study consisted of 229 infertile Spanish women and 236 infertile Indian women undergoing their first or second IVF cycles. Spanish patients were monitored at IVI Madrid, and Indian patients were followed at Nova IVI Fertility clinics in Delhi and Ahmedabad.

Inclusion and Exclusion Criteria

To include a homogeneous population, the criteria for inclusion in this study were age $\leq$42 years, both ovaries present, and undergoing IVF treatment because of male-factor infertility, tubal disease, or previous failed intrauterine insemination. Women diagnosed with polycystic ovary syndrome according to the Rotterdam criteria, endometriosis stage III–IV, previous adnexal surgery, or pelvic inflammatory disease were excluded.

Patients provided written informed consent, and Institutional Review Board (IVI-Madrid) approval from our institution was obtained before initiation of the study (MAD-CI-11-2012-01). Data were gathered anonymously to avoid individual patient identification, as consistent with data protection rules in our institution.

Treatment Protocol

Patients underwent conventional COS as previously described (14). Briefly, patients received a starting dose of recombinant FSH (Puregon, Organon; or Gonal F, Serono) or highly purified hMG (Menopur, Ferring) ranging from 150 IU to 225 IU; 0.25 mg GnRH antagonist Ganirelix (Orgalutran, Organon) was administered daily starting on day 5 or 6. The cycle was monitored according to the policy of the patient's clinic. Recombinant hCG (Ovitrelle, Serono) was administered as soon as two leading follicles reached $\geq$17 mm mean diameter, and oocyte pickup was performed 36 hours later. Fertilization was performed with conventional IVF or intracytoplasmic sperm injection (ICSI), according to individual criteria.

Data collected included age, body mass index (BMI), ovarian reserve markers (day 3 serum FSH, and E_2, and AMH levels), and AFC via transvaginal ultrasonography. We also collected IVF results after COS for both ethnic groups, including the amounts of gonadotropin used, the number of oocytes retrieved, the number of mature oocytes, the number of oocytes fertilized, and the number of embryos obtained.

AMH levels were measured in patient serum. Blood was collected by peripheral venipuncture; the serum was separated from cells by centrifugation, and samples were frozen at -20°C until assayed. Immunoreactive AMH concentrations were determined with the use of ELISA (Immunotech; Beckman Coulter) according to the manufacturer's instructions. All samples were tested in the same assay and performed in duplicate. The intra-assay and interassay coefficients of variation were 12.3% and 14.2%, respectively (15).

We followed the recommendations of Brokemans et al. (16) to correctly measure antral follicles. With the use of a real-time two-dimensional transvaginal transducer, we counted follicles from days 2 to 4 of the cycle to avoid the effect of intracycle variation. Our counts included all antral follicles 2–10 mm in diameter.

We used the following systematic process to count antral follicles: 1) Identify the ovary; 2) explore the dimensions in two planes (perform a scout sweep); 3) decide on the direction of the sweep to measure and count follicles; and 4) measure the largest follicle in two dimensions. If the largest follicle was $\leq$10 mm in diameter, we started to count from the outer ovarian margin of the sweep to the opposite margin, considering every round or oval transonic structure within the ovarian margins to be a follicle. We repeated this procedure with the contralateral ovary, and combined the number of follicles in each ovary to obtain the AFC. If the largest follicle was $>$10 mm in diameter, we further ascertained the size range of the follicles by measuring each sequentially smaller follicle until a follicle with a diameter $\leq$10 mm was found. We then performed a total count (as described) regardless of follicle diameter, and subtracted the number of follicles $>$10 mm from the total follicle count to determine the AFC (16).

Statistical Analysis

Because no previous data existed to take as a reference for our sample size calculations, we decided to evaluate 200 patients per arm, considering that the data obtained from these women would be clinically relevant.

Categoric data are expressed as number and percentage, and continuous data are expressed as mean $\pm$ SD. Continuous variables were examined for normality with the Kolmogorov-Smirnov test; if the data were not normally distributed, nonparametric tests were used. Statistical analyses were performed with the chi-square test, Fisher exact test, or two-sample Student *t* test. Linear regression analysis was used to assess the association between variables. To control for confounding factors, stratification was undertaken for: cause of infertility, age, BMI, and duration of infertility. Factors having an impact on ovarian reserve were assessed with the use of a multivariate analysis that included the following potential related factors: age, AFC, AMH, basal FSH, and number of