ORIGINAL ARTICLE: ENDOMETRIOSIS

Patients with endometriosis have aneuploidy rates equivalent to their age-matched peers in the in vitro fertilization population

Caroline Juneau, M.D.,[a,b] Emily Kraus, M.D.,[c] Marie Werner, M.D., H.C.L.D.,[a] Jason Franasiak, M.D., T.S.,[a,b] Scott Morin, M.D.,[a,b] George Patounakis, M.D., Ph.D.,[d] Thomas Molinaro, M.D., M.S.C.E.,[a] Dominique de Ziegler, M.D.,[e] and Richard T. Scott, M.D., H.C.L.D.[a,b]

[a] Reproductive Medicine Associates of New Jersey, Basking Ridge, New Jersey; [b] Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania; [c] Medical University of South Carolina, Charleston, South Carolina; [d] Reproductive Medicine Associates of Florida, Lake Mary, Florida; and [e] Hôpital Cochin, University Paris Descartes, Paris, France

Objective: To determine whether endometriosis ultimately results in an increased risk of embryonic aneuploidy.
Design: Retrospective cohort.
Setting: Infertility clinic.
Patient(s): Patients participating in an in vitro fertilization (IVF) cycle from 2009–2015 using preimplantation genetic screening (PGS) who had endometriosis identified by surgical diagnosis or by ultrasound findings consistent with a persistent space-occupying disease whose sonographic appearance was consistent with endometriosis.
Intervention(s): None.
Main Outcome Measure(s): Rate of aneuploidy in endometriosis patients undergoing IVF compared to controls without endometriosis undergoing IVF.
Result(s): There were 305 patients with endometriosis who produced 1,880 blastocysts that met the criteria for inclusion in the endometriosis group. The mean age of the patients with endometriosis was 36.1 ± 3.9 years. When the aneuploidy rates in patients with endometriosis and aneuploidy rates in patients without endometriosis were stratified by Society for Assisted Reproductive Technology age groups and compared, there were no statistically significant differences in the rate of aneuploidy (odds ratio 0.85; 95% confidence interval, 0.84–0.85).
Conclusion(s): Patients with endometriosis undergoing IVF have aneuploidy rates equivalent to their age-matched peers in IVF population who do not have endometriosis. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Aneuploidy, endometriosis, in vitro fertilization, preimplantation genetic screening

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Endometriosis, which affects nearly 30% of infertile patients (1), is associated with poor in vitro fertilization (IVF) outcomes, including a decreased yield of mature oocytes (2), lower implantation rates (3, 4), and decreased pregnancy rates (3–5). Although these outcomes are well documented, the mechanism for this lack of success and the associated morbidities has yet to be clearly elucidated. It is likely a combination of multiple pathologic pathways, including possible alterations in the meiotic spindle that could affect the rate of aneuploidy in patients affected by endometriosis and contribute to the reduced outcomes seen in this population (6, 7).

During meiosis, complete nuclear maturation is critically dependent on normal spindles to guide chromosomal segregation and organization (8, 9). In endometriosis, alterations of the meiotic spindle apparatus have been described, raising the question of whether the oocytes in endometriosis patients are more susceptible to

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Reprint requests: Caroline Juneau, M.D., Reproductive Medicine Associates of New Jersey, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Sidney Kimmel Medical College, Thomas Jefferson University, 140 Allen Road, Basking Ridge, New Jersey 07920 (E-mail: cjuneau@rmanj.com).

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meiotic errors and chromosomal instability (10–12). This in turn could lead to an increase in aneuploidy. Given these findings, increased attention has been paid to the investigation of the spindle apparatus in oocytes, particularly in endometriosis (6, 7, 13, 14).

In animal models, Mansour et al. (13) illustrated that peritoneal fluid isolated laparoscopically from patients with endometriosis causes microtubule damage in murine oocytes. In this study, increased oocyte anomalies and embryo apoptosis were observed. Da Broi et al. (6) performed a more applicable assessment in which bovine oocytes underwent in vitro maturation in the follicular fluid of women with and without endometriosis and reported similar findings. A statistically significantly higher rate of meiotic abnormalities was seen in the oocytes matured in the follicular fluid of endometriosis patients (6).

To date, no study has investigated whether altered spindle dynamics can be translated into an increased risk of aneuploidy in patients with endometriosis. There have been valuable contributions to the study of plausible explanations for poor outcomes in endometriosis patients, but they were limited in that few studies have involved human oocytes or used the culture systems typically used by IVF laboratories. Furthermore, they do not directly measure a clinical outcome. We examined the rates of aneuploidy in endometriosis patients compared to the general IVF population.

MATERIALS AND METHODS

Study Population

Our retrospective analysis involved all couples undergoing IVF with preimplantation genetic screening (PGS) between 2009 and 2015. The patients were separated into two groups: those who had evidence of endometriosis (experimental group) and those who did not (control group) to compare the rates of aneuploidy. The diagnosis of endometriosis was assigned if patients had evidence of disease at the time of surgery or in symptomatic women if ultrasound findings demonstrated persistent space-occupying disease consistent with endometriosis.

Demographic information and markers of ovarian reserve were collected, including age (years), minimum antimüllerian hormone (AMH) level (μg/L), and maximum day-3 follicle-stimulating hormone (FSH) level (mIU/mL). Cycle responses—including number of oocytes retrieved, fertilization rates, blastulation rates, and surge estradiol (pg/mL) levels—were compared between the two groups. The experimental group and the control group were then divided for analysis into Society for Assisted Reproductive Technology (SART) age categories: $<$35 years, 35–37 years, 38–40 years, 41–42 years, and $>$42 years. Additionally, age was treated as a continuous variable and controlled for in a subsequent analysis. Approval for the study was obtained by the Schulman Institutional Review Board (Research Triangle Park, NC) under protocol 201505698.

Oocyte Retrieval and PGS

The IVF cycle was performed per practice routine. Multifollicular ovarian stimulation was achieved with highly purified urinary gonadotropins: FSH (recombinant and urinary) and menotropins. Final oocyte maturation was typically induced with 5,000 to 10,000 IU of human chorionic gonadotropin when at least two follicles had reached 18 mm in maximal diameter. Transvaginal oocyte retrieval was performed 36 hours after human chorionic gonadotropin administration.

Fertilization was achieved with intracytoplasmic sperm injection in all cases owing to the intent to perform PGS. Normally fertilized zygotes were cultured in cleavage medium in a low-oxygen tension environment. On day 3 of embryo development, all cleaved embryos underwent laser-assisted hatching of the zona pellucida to facilitate trophectoderm biopsy. All embryos were then placed in extended culture regardless of the size or quality of the cohort. All usable blastocysts underwent PGS. A blastocyst was considered usable if it was suitable for transfer or vitrification for future use.

Statistical Analysis

The use of Student's *t*-test or the Mann-Whitney *U* test were applied to parametric and nonparametric data, respectively, to compare baseline characteristics and cycle outcomes between the groups. Pearson's chi-square test with Yate's continuity correction was used for comparing the proportion of euploid embryos within each group across SART age categories.

Embryos within a given patient's cohort were initially assumed to be independent for the SART age group analysis because female age has the largest effect on degree of correlation in any given patient's embryo cohort. Subsequently, a mixed-effects logistic regression model employing a random effect at the patient level was constructed to account for the residual correlation between embryos from the same patient while controlling for the confounder of age.

Age was accounted for in two different ways to better approximate its effects on aneuploidy. First, it was treated as a simple linear predictor with a single coefficient in the model. Then age was modeled as a piecewise linear function with a different coefficient (slope) for patients younger than 36 years and those older than 36 years to better approximate the nonlinearity of aneuploidy with age.

All statistical calculations were performed with SPSS version 23 (IBM) while post hoc power calculations were performed with G*Power version 3.1.9.2 (Universität Düsseldorf). An alpha error of less than 0.05 was considered statistically significant.

RESULTS

In the endometriosis group 1,880 blastocysts from 305 patients were analyzed, and 23,054 blastocysts from 3,798 patients were included in the control group. The mean age of the endometriosis patients was 36.1 $\pm$ 3.9 years. Their median antimüllerian hormone (AMH) concentration was 1.30 ng/mL (interquartile range [IQR] 0.68–2.87 ng/mL), which was lower than the general IVF population as would be expected. The mean FSH level was 7.7 $\pm$ 3.2 mIU/mL, which was equivalent between the two groups. Per endometriosis patient, there were 12 oocytes (IQR 8–17) retrieved, and 7 oocytes (IQR 4–11) were successfully fertilized. These cycle parameters were

TABLE 1

Baseline characteristics and in vitro fertilization (IVF) cycle outcomes among endometriosis patients and the IVF population without endometriosis (control group).

Characteristic	Control	Endometriosis	*P* value
Age (y)	36.3 ± 4.4	36.1 ± 3.9	.773[a]
BMI (kg/m^2)	23.9 (21.4–27.9)	23.3 (21.1–26.4)	.019[b,c]
Minimum AMH	1.8 (0.85–3.58)	1.3 (0.68–2.87)	<.001[b,c]
Maximum day-3 FSH	7.2 ± 3.0	7.7 ± 3.2	.073[a]
E_2 day of surge	1,866 (1,219–2,722)	1,854 (1,278–2,716)	.950[b]
No. oocytes retrieved	13 (8–20)	12 (8–17)	.014[b,c]
No. of 2PN	8 (5–12)	7 (4–11)	.040[b,c]
No. of usable blastocysts	4 (2–7)	3 (2–6)	.054[b]
PGS platform			.754[d]
PCR	3,389/3,798 (89.2%)	277/305 (90.8%)	
NGS	242/3,798 (6.4%)	16/305 (5.2%)	
Microarray	167/3,798 (4.4%)	12/305 (3.9%)	

Note: AMH = antimüllerian hormone; BMI = body mass index; E_2 = estradiol; FSH = follicle-stimulating hormone; NGS = next-generation sequencing; PCR = polymerase chain reaction; PGS = preimplantation genetic screening; 2PN = two pronuclei.
[a] Student's *t*-test.
[b] Mann-Whitney *U* test.
[c] Statistical significance, *P*< .05.
[d] Chi-square test.

Juneau. No higher rate of aneuploidy in endometriosis. Fertil Steril 2017.

slightly lower in endometriosis patients as expected, but the number of usable blastocysts formed was equivalent in comparing the endometriosis patients and the control group (3; IQR 2–6; *P*=.054). These data also demonstrate that the experimental group and control group were well matched by age and use of varying PGS platforms (Table 1).

Of the embryos from endometriosis patients aged <35 years, 179 (25.9%) of 690 blastocysts were aneuploid; in the control group, 2,545 (25.2%) of 10,117 blastocysts were aneuploid (*P*=.678; Table 2; Fig. 1). In patients aged 35–37 years, 182 (34.0%) of 536 blastocysts from the endometriosis group were aneuploid, and 1,011 (35.4%) of 5,674 from the control group (*P*=.521). In the women aged between 39 and 40 years, 156 (52.2%) of the 299 endometriosis patients' blastocysts and 1,562 (48.6%) of the 3,214 control patients' embryos were aneuploid (*P*=.262). For the women aged between 41 and 42 years, 176 (62.4%) of 282 blastocysts were aneuploid from the endometriosis patients and 1,633 (62.1%) of 2,629 from the control group (*P*=.974). For patients older than 42 years, 62 (84.9%) of 73 blastocysts from the endometriosis group were aneuploid and 1,169 (82.3%) of 1,420 from the control group (*P*=.679).

As demonstrated here, there were no statistically significant differences in aneuploidy between the patients with endometriosis and the control population after stratifying by age. This study had 80% power to detect between a 5% to 9% difference in aneuploidy between those with endometriosis and those without across the SART age groups given an alpha error of 0.05.

To determine whether other unmeasured patient factors were affecting the results of the analysis, such as correlation between embryos from the same patient, a mixed-effects logistic regression was performed (Table 3). This analysis concurs with the results of the stratification by SART age group analysis in showing endometriosis does not increase the odds of obtaining an aneuploid embryo in the unadjusted

TABLE 2

Embryonic sample size: rate of aneuploidy is not increased in endometriosis patients when compared with age-matched controls.

SART age group (y)	No. of euploid embryos		
	Control	Endometriosis	*P* value
<35	7,572/10,117 (74.8%)	511/690 (74.1%)	.678
35–37	3,663/5,674 (64.6%)	354/536 (66.0%)	.521
38–40	1,652/3,214 (51.4%)	143/299 (47.8%)	.262
41–42	996/2,629 (37.9%)	106/282 (37.6%)	.974
>42	251/1,420 (17.7%)	11/73 (15.1%)	.679

Note: P values are based on the chi-square test. SART = Society for Assisted Reproductive Technology.

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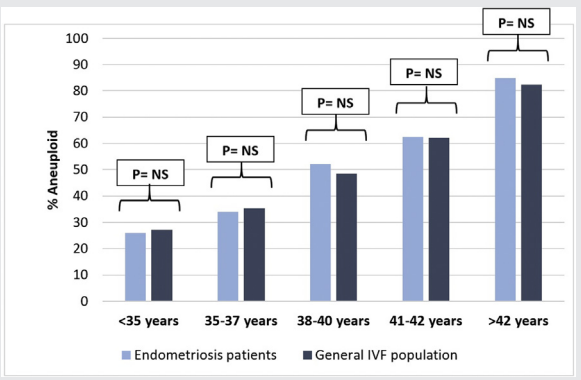
FIGURE 1



In women undergoing in vitro fertilization, the rate of aneuploidy in the embryos of endometriosis patients did not differ from that of women without endometriosis.

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TABLE 3

Mixed effects logistic regression: rate of aneuploidy no difference in endometriosis patients undergoing in vitro fertilization (IVF) as compared with the general IVF population but influenced by age.

Model	Effect of endometriosis vs. healthy patients: odds of euploid embryo (no endometriosis as reference)	Endometriosis effect, *P* value	Effect of age per increasing year on odds of euploid embryo	Age effect, *P* value
Unadjusted model	0.96 (0.84–1.1)	.582	NA	NA
Adjusted for age	1.02 (0.91–1.1)	.747	0.85 (0.84–0.85)	<.001
Adjusted for age with two coefficients	0.99 (0.88–1.1)	.837	0.95 (0.93–0.96) for age <36 y 0.76 (0.75–0.77) for age ≥36 y	<.001 <.001

Note: NA = not applicable.

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odd, adjusted odds with age as a linear predictor, and adjusted odds with age as modeled in a piecewise linear fashion.

Discussion

It is known that women with endometriosis have lower rates of success during IVF treatment (4). Data have shown that the spindle cell complex of oocytes in women with endometriosis could be altered and may be prone to microtubule compromise and increased meiotic errors (2, 6, 13). This could explain an increased risk for aneuploidy in endometriosis patients; however, in our data, the aneuploidy rates in endometriosis patients were found to be equivalent to those of patients in the general IVF population with no evidence of endometriosis.

Using human oocytes, Barcelos et al. (14) performed a prospective analysis including germinal vesicles and metaphase 1 oocytes from patients with endometriosis and control patients. The oocytes were fixed and processed for meiotic spindle evaluation using immunofluorescence microscopy. Contrary to the afore mentioned animal studies, no differences in the meiotic spindle apparatus were appreciated in their analysis. They did note a tendency toward a higher proportion of telophase I oocytes in endometriosis patients, suggesting there may be impairment in the completion of meiosis I, which would require further evaluation. Additionally, the in vivo maturation of oocytes of both endometriosis and unaffected patients were studied by Dib et al. (7). Both groups of oocytes showed similar rates of nuclear maturation as well as spindle location and visualization, a marker of spindle normalcy, thus adding to the debate about the mechanism of compromised IVF outcomes.

Conflicting data in the literature suggest that the pathogenesis of poor reproductive outcomes observed in endometriosis patients is multifactorial. It has been suggested that women with endometriosis have increased rates of granulosa cell apoptosis (15) as well as increased concentrations of follicular fluid natural killer cells and lymphocytes, implicating altered immune function (16) as a proposed mechanism for these sequelae. Endometrial defects have also been suggested (17), including reduced endometrial receptivity. This has been described in women with endometriosis and could be due to delayed histologic maturation. Biochemical alterations in the endometrial environment have been demonstrated with reduced or absent concentrations of the cellular adhesion molecule $\alpha v\beta 3$ integrin in the endometrium of affected women (18). There may also be impaired interactions between the endometrium and the ovary itself (19).

A potential limitation to our findings is that the group of experimental patients includes those patients with either a surgical diagnosis of endometriosis or an ultrasound diagnosis of persistent space-occupying disease. Therefore, this study is unable to stratify aneuploidy outcomes based on the stage or severity of endometriosis, as not all patients had laparoscopic assessment of disease. The endometriosis patients included in this analysis likely had moderate- to advanced-stage disease. This approach, including women in whom endometriosis is diagnosed by imaging, is necessary now that surgery is more rarely performed—including when endometriosis exists—for fear that it might harm ovarian reserve (20). This approach is further supported by the description of new systematic approaches for diagnosing endometriosis by ultrasound (21).

The selection criteria, while challenging, were necessary to ensure that the study population would include patients with moderate- to advanced-stage (stage 3 and 4) endometriosis but would inevitably miss patients with less severe disease. There are also likely undiagnosed patients that this approach would not include, who may be included in the control group as many patients with endometriosis do not undergo surgery to confirm their diagnosis.

Despite the struggle to capture varying levels of disease severity frequently faced in the study of endometriosis, the stringent criteria for inclusion in the study population include a reliable experimental group affected by disease; should a difference in aneuploidy be found, it would only strengthen the conclusion. Finally, this approach also benefited the validity of the control group who had no evidence of endometriosis.

In our study, only usable blastocysts are included in this analysis. If alterations in the spindle apparatus resulted in developmental arrest before the blastocyst stage, those embryos would not have been included in the analysis.

Prior studies were inconclusive, and small sample sizes limited many of their findings. Our is the first study to analyze whether endometriosis patients are at increased risk of aneuploidy, a clinically relevant outcome. The extent of the embryonic sample size in our data for both the control and the

experimental group strengthens our findings. As anticipated, the rate of aneuploidy increases with advancing maternal age, regardless of the diagnosis of endometriosis. These data demonstrate that the rate of aneuploidy is not statistically significantly different between patients with endometriosis as compared with age-matched controls in the IVF population.

Conclusion

Our analysis found that women with endometriosis undergoing IVF are not at an increased risk of aneuploidy. It is likely that the reduced success in assisted reproduction that has been observed in endometriosis patients involves multiple pathologic mechanisms.

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