

# Novel ploidy analysis in ectopic pregnancy

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**Objective:** To study whether a single-nucleotide polymorphism (SNP) array could be used to test tissue from ectopic pregnancy to distinguish whether ectopic pregnancies were aneuploid.

**Design:** Case series report.

**Setting:** Academic medical center.

**Patient(s):** One hundred seventy-eight women who underwent surgery for ectopic pregnancy at Northwestern Memorial Hospital between 2015 and 2018 were eligible for participation; written consent was obtained from 33 patients. Eight subjects had sufficient DNA samples and were included in the analysis. Maternal and paternal DNA samples were self-collected by buccal swab. Archived paraffin tissue containing chorionic villi from each surgically removed ectopic specimen was analyzed using SNP microarray technology to determine chromosome number and evaluate for maternal cell contamination.

**Intervention(s):** None.

**Main Outcome Measure(s):** Prevalence of aneuploidy in ectopic pregnancy specimens as well as success of SNP array technology in formalin-fixed and paraffin-embedded specimens.

**Result(s):** Subjects had a mean ( $\pm$ SD) age of  $33.4 \pm 5.4$  years, body mass index of  $23.4 \pm 5.7$  kg/m<sup>2</sup>,  $3.3 \pm 1.8$  prior pregnancies, and  $1.5 \pm 1.4$  live births. Genetic testing revealed that all eight tested samples were euploid, 6 female and 2 male (two arr(1-22)x2, (X,Y)x1 and 6 arr(1-22, X)x2); maternal cell contamination was ruled out in all cases.

**Conclusion(s):** This study showed proof of concept for the use of routinely stored formalin-fixed, paraffin-embedded tissue blocks with DNA extraction for SNP array to detect ploidy status of ectopic pregnancy. Although all tested samples were euploid, further research is needed to gain a definitive answer to this question and better understand the mechanism that leads to ectopic implantation. (Fertil Steril Rep® 2021;2:67-71. ©2020 by American Society for Reproductive Medicine.)

**Key Words:** Ectopic pregnancy, SNP array, aneuploidy, implantation

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Since the advent of single-nucleotide polymorphism (SNP) array technology, its use has expanded throughout the field of obstetrics and gynecology (1). In the field of reproductive endocrinology, SNP array testing has allowed for more precise identification of aneuploidy with

improved accuracy through identification of maternal cell contamination. This technology has been widely applied in assessing aneuploidy in spontaneous abortions (2-4).

SNP array has also been used to evaluate aneuploidy in preimplantation genetic testing. The use of this

technology and others, such as comparative genomic hybridization arrays, has allowed confirmation that chromosomal abnormalities in preimplantation embryos are higher than in spontaneous abortions (5). This indicates a selection mechanism whereby many aneuploid embryos do not implant and are prevented from further development. Some studies have demonstrated that tested euploid embryos have higher implantation rates than untested embryos (65.6% vs. 46.9%), whereas other studies have failed to show a benefit (5, 6).

Ectopic pregnancy is a cause of significant morbidity and mortality and accounts for 2.7% of all pregnancy-related deaths (7). Despite the known risk factors of pelvic infection, prior pelvic surgery, smoking,

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and assisted reproductive technology, 50% of ectopic pregnancies remain unexplained (8, 9). Others have attempted to extrapolate aneuploidy as a common risk factor for both spontaneous miscarriage and ectopic pregnancy. It previously was established that fetal karyotype abnormality was the most common cause of pregnancy loss (10). Job-Spira et al. (11) reported that women with recurrent ectopic pregnancies were significantly more likely to also have had previous pregnancy losses, and they concluded that chromosomal abnormalities could offer a link between these two associated phenomena.

Although the use of archived pathology slides with routinely stored formalin-fixed, paraffin-embedded (FFPE) tissue blocks has been previously described to assess aneuploidy status in the setting of a previous spontaneous abortion, the use of this methodology and technology has not yet been described in the setting of an ectopic pregnancy. The question of aneuploidy as a contributory factor in ectopic pregnancy has been a source of debate for nearly half a century. Given the association between ectopic pregnancy, recurrent pregnancy loss, and the known association between age and aneuploidy, we explored whether the use of routinely stored FFPE tissue blocks with DNA extraction and SNP-based array technology could be used to assess ploidy status of surgically removed ectopic pregnancies.

## MATERIALS AND METHODS

### Study Design

Institutional Review Board (IRB STU00203775) approval was obtained. This was a single-center, retrospective clinical study performed in the Division of Reproductive Endocrinology and Infertility at Northwestern University. An Electronic Data Warehouse search was conducted to identify all women who underwent surgical removal of an ectopic pregnancy by salpingectomy or salpingostomy at Northwestern Memorial Hospital from January 2015 to December 2018. Eligible subjects were contacted, and written informed consent was obtained. Follow-up calls were made to patients who did not return consent forms. Maternal and paternal DNA samples were self-collected by buccal swab.

### Genetic Analysis

Specimens were stored at Northwestern Memorial Hospital for up to 4 years on site and then moved to a secure warehouse for at least 10 years before destruction. All samples of ectopic pregnancies at Northwestern are routinely processed and fixed in paraffin within 48 hours after surgery. Archived paraffin tissue was examined for the presence of chorionic villi within each surgically removed ectopic pregnancy specimen. Paraffin tissue samples were submitted as slides including: one hematoxylin and eosin stained slide and nine unstained paraffin slides per case. Parental buccal samples were submitted according to the referral laboratory collection protocol. Fetal DNA was identified on the stained slide and dissected from the unstained slide sections. DNA was extracted from paraffin samples using a kit (REPLI-g FFPE kit; Qiagen, Germantown, MD). In this kit, the reagents

for preparing fragmented DNA from paraffin-embedded tissue for amplification are used to ligate DNA fragments in a random order. Then, the reagents containing DNA polymerase (REPLI-g Midi; Qiagen) are added, which provides uniform amplification across the entire genome (12). The kit is unable to process DNA segments <500 bp in length or when a small number of genome equivalents are used (12).

DNA was extracted from parental samples using the DNA extraction solution (QuickExtract; Lucigen, Middleton, WI). After whole genome amplification, purified DNA from the parental and fetal tissue was analyzed using the genotyping microarray platform (CytoSNP-12; Illumina, San Diego, CA) with approximately 300,000 probes covering all 24 chromosomes (1-22, X, Y). Results were analyzed using algorithms (Parental Support; Natera, San Carlos, CA). This analyzes chromosome copy number, uniparental disomy, and parental origin of aneuploidy. Tissue sample genetic results were compared with maternal and paternal SNP genotype information through bioinformatic algorithms that evaluate for maternal cell contamination, confirm chromosome content, and determine parental source of each chromosome in a specimen. This analysis cannot detect chromosomal duplications, deletions, or balanced tetraploidy or differentiate between maternal triploidy and maternal cell contamination.

## RESULTS

One hundred seventy-eight women were eligible for participation and were contacted. Thirty-three subjects enrolled and their written informed consent was obtained. Parental DNA samples were available for 24 subjects. Sixteen of the 24 samples could not be analyzed because they lacked sufficient embryonic DNA. Eight subjects, who had surgical removal via laparoscopic salpingectomy, had samples with sufficient DNA and were included in the final analysis (Table 1).

The subjects with samples that had sufficient DNA had a mean ( $\pm$ SD) age of  $33.4 \pm 5.4$  years at the time of surgery, body mass index (BMI) of  $23.4 \pm 5.7$  kg/m<sup>2</sup>,  $3.3 \pm 1.8$  prior pregnancies, and  $1.5 \pm 1.4$  live births. Electronic medical records were reviewed, and none of the subjects had a history of smoking, pelvic inflammatory disease, sexually transmitted infection (STI), assistive reproductive technology, infertility, or prior ectopic pregnancy. The women whose samples had insufficient DNA for analysis had a mean age of  $33.1 \pm 4.9$  years and a mean BMI of  $23.1 \pm 2.7$  kg/m<sup>2</sup>; they were not significantly different from the women whose samples had sufficient DNA for analysis. Among the women with samples with insufficient DNA, six had a prior pregnancy, one was a former smoker, one had a history of STI, three had a history of infertility, and two had a prior ectopic pregnancy.

Genetic testing revealed that 100% (8/8) of the included ectopic pregnancies were euploid, 95% confidence interval 62.8%, 100%. Two of the tested specimens were male and six were female (two arr(1-22)x2, (X,Y)x1 and six arr(1-22, X)x2) (Table 2). Maternal cell contamination was ruled out in all cases.

TABLE 1

Patient characteristics.	
Demographics	Ectopic Pregnancies
Mean age, y (SD)	33.4 (5.01)
Mean BMI, kg/m <sup>2</sup> (SD)	23.4 (5.70)
Mean number of prior pregnancies (SD)	2.3 (1.8)
Mean number of live births (SD)	1.5 (1.4)
Patient History, % (n/N)	
History of Smoking	0 (0/8)
History of pelvic inflammatory disease	0 (0/8)
History of sexually transmitted disease	0 (0/8)
History of infertility	0 (0/8)
History of prior ectopic	0 (0/8)

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## DISCUSSION

The use of FFPE tissue extraction for analysis has been implemented widely, notably in the field of obstetrics and gynecology in cancer genomics research, molecular genotyping of illnesses such as human papillomavirus, and for genetic analysis in intrauterine pregnancy loss (4, 13, 14). FFPE tissue analytics have the advantage of retrospective queries from previously collected tissue (13).

This is the first study to report the use of SNP microarray technology to analyze the ploidy status of a prior ectopic pregnancy from tissue stored in formalin as a fixed paraffin-embedded sample. SNP testing with the Parental Support algorithm analyzes DNA fingerprints of the ectopic specimen and compares this to the maternal and paternal DNA samples. This allows investigators to rule out maternal cell contamination and identify the parental origin of aneuploidy. SNP array analysis does not require living tissue and can be performed with a low input of genomic DNA thus making it a feasible method for paraffin-embedded tissue analysis.

Despite the advantages of SNP array technology, over half of all analyzed samples in the present study were found to have insufficient fetal DNA for analysis. This rate is lower than the previously reported 71% and 86.4% success rates from FFPE samples in studies on intrauterine pregnancies (4, 15). DNA extraction can be limited by both small amounts of DNA as well as the quality of the sample, which is influenced by the time of formalin fixation, storage conditions, and purification method used. In this study, as samples were retrospectively investigated nearly 5 years later, quality control was not assured given the length of storage and the unknown quality of storage once the samples reached the warehouse. Additionally, the small amount of fetal DNA in these samples can be difficult to identify and extract. This difficulty in obtaining DNA contributed to a smaller than expected sample size. In future studies, several lessons can be applied to improve DNA extraction and thus result in more meaningful ploidy analysis. Laboratory techniques can be optimized for DNA extraction; these include trimming the

TABLE 2

Genetic testing results of eight ectopic pregnancies.	
Case	Result
Case 1	arr(1-22, X)x2
Case 2	arr(1-22)x2, (X,Y)x1
Case 3	arr(1-22, X)x2
Case 4	arr(1-22, X)x2
Case 5	arr(1-22)x2, (X,Y)x1
Case 6	arr(1-22, X)x2
Case 7	arr(1-22, X)x2
Case 8	arr(1-22, X)x2

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tissue to eliminate most of the paraffin if the tissue is <1 cm in diameter, using less FFPE lysis solution, and freshly cutting the starting material for DNA application (12). Additional strategies include immersing the tissue in cold, buffered formalin and ensuring it is brought to pathology within 48 hours to reduce the warm ischemia time (13). Furthermore, future studies could be prospective with tissue collected at the time of surgery for ectopic pregnancy and avoid the limitations inherent to DNA extraction from FFPE samples. The discrepancy in DNA tissue extraction success between intrauterine and extrauterine pregnancy tissue suggests that ectopic pregnancies have unique characteristics that may present challenges for tissue extraction, such as having less tissue available for analysis given the gestational age at ectopic presentation or rupture. Future studies could investigate the correlation of salpingectomy versus salpingostomy and even of human chorionic gonadotropin level on the success of FFPE DNA extraction.

In addition, this study was limited by the inability of the technology to detect duplications, deletions, or balanced tetraploidy, and with the use of this technology maternal triploidy would be reported as maternal cell contamination. Additional limitations may include the inability to detect mosaicism or understand the impact of mosaicism on ectopic implantation. A prospective analysis using fresh tissue samples collected at the time of surgery with parental blood samples would optimize both the ability to obtain DNA and to detect deletions, duplications, maternal triploidy, and mosaicism.

Since the 1970s, investigators have explored the relationship between ectopic pregnancy and embryonic aneuploidy. However, early studies were limited by the analysis technique, with limited culture success rates (16). Additionally, the rate of abnormal karyotype in these studies ranged from 4%–78%, making definitive conclusions difficult (17, 18). A literature review for the present study revealed 13 English-language articles that attempted to characterize the prevalence of aneuploidy among ectopic pregnancies (Table 3) with a cumulative summary of 22.5% of ectopic pregnancies reported to be aneuploid. In our current analysis, the aneuploidy rate was 0% but the 95% confidence interval of 0% to 37.2% does overlap previous estimates.

The SNP array was used in this study not only to apply a novel technology in this setting but also to determine if less

TABLE 3

Literature review results.				
Study, y	No. of Samples	Genetic Analysis Technique	Sex	Aneuploidy Rate
Busch, 1974(19)	25	Karyotype	14 XX 9 XY 2 XXXX	16% (4/25)
Poland, 1976(16)	16	Karyotype	6 XX 8 XY 1 XXXY 1 tetraploid unable to be characterized	31.3% (5/16)
Elias, 1981(20)	23	Karyotype	14 XX 7 XY 1 XXXX 1 XXX	17% (4/23)
Aine, 1990(21)	42	Flow Cytometry	Not reported	33% (14/42)
Cohen, 1993(22)	60	Karyotype	Not reported	78% (47/60)
Karikoski, 1993(23)	42	Flow Cytometry	Not available	33% (14/42)
Toikkanen, 1993(24)	55	Flow Cytometry	Not reported	23.6% (13/55)
Goddjin, 1996(25)	22	Karyotype	6 XX 15 XY 1 XXX	4.5% (1/22)
Erel, 1996(26)	12	Flow cytometry	Not reported	38.5% (5/12)
Block, 1998(17)	21	Karyotype	12 XX 7 XY 1 XXY 1 X	14.3% (3/21)
Coste, 2000(18)	62	Karyotype	35 XX 26 XY	4.8% (3/62)
Goddjin, 2005(27)	54	Quantitative Fluorescent PCR (analysis restricted to chromosomes 16, 18, 21, X and Y)	Not reported	3.7% (2/54)
Furuya, 2017(28)	88	Karyotype	42 XX 46 XY	3.4% (3/88)
Overall Total	574	Various		22.6% (118/522)

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sophisticated genetic analysis methods were underestimating the rate of aneuploidy in historical studies. For example, conventional cytogenetic analysis by karyotype requires that chorionic villi are separated from the maternal tissue and grown in culture. Although this method is readily available, it must be performed on living tissue and has a high rate of culture failure. Flow cytometry has the advantage of rapid and simple testing on previously fixed tissue in paraffin wax (21). However, the technique is limited by its inability to demonstrate small DNA rearrangements. One article in our review used quantitative fluorescent polymerase chain reaction, although the investigators assessed only chromosomes 16, 18, 21, X, and Y (25). This has the potential for significantly underestimating the aneuploidy rate within the sample as ectopic pregnancies may have a wide range of chromosomal abnormalities.

This present study suggests a lower rate of aneuploidy than previously described. However, given the small sample size, no definitive conclusions can be made. The presence of euploid pregnancy tissue in this study does render the statement that all ectopic pregnancies have abnormal ploidy status as false, because SNP-based microarray technology revealed a normal number of chromosomes in all collected ectopic pregnancy specimens. In addition, the subjects had no known

risk factors for ectopic pregnancy, such as a history of pelvic inflammatory disease, STI, smoking, or prior ectopic pregnancy. Other factors, such as hormonal changes, toxin exposures, and endometrial thickness may be causative (29–32). The lower rate of aneuploidy reported here may be an indicator that aneuploidy is not a strong risk factor for the etiology of ectopic pregnancy, or that previous studies with less sophisticated genetic analysis may have overestimated aneuploidy rates. However, we have successfully demonstrated that SNP array technology can be used to assess ploidy status in ectopic pregnancy.

SNP array has been extensively used for the diagnosis of aneuploidy in spontaneous abortions, but the use of this technology in determining ploidy status of ectopic pregnancy has never been described previously. In this study, all ectopic pregnancy samples were euploid and maternal cell contamination was ruled out. Further studies with more subjects and fresh tissue samples or application of optimal strategies for FFPE DNA extraction are needed to further explore whether aneuploidy is a risk factor for ectopic implantation.

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