Consistency of endometrial receptivity array and histologic dating of spatially distinct endometrial samplings: a prospective, blinded study

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Objective: To compare the consistency of endometrial receptivity array (ERA) and histologic dating among 3 spatially distinct endometrial samples obtained during a cycle of exogenous estrogen and progesterone.
Design: Prospective blinded study.
Setting: University practice.
Patients: Twelve patients undergoing a mock frozen embryo transfer cycle.
Intervention: Endometrial biopsy was performed in a manner that provided a spatially organized endometrial specimen, corresponding to the fundus, middle, and lower segment. Each of these 3 sections was further divided into immediately adjacent specimens for ERA and histology.
Main Outcome Measure: Consistency of the ERA and histology results among fundal, mid, and lower endometrial biopsy specimens.
Results: The ERA showed variability in outcome among different patients but dated all specimens originating from the same patient identically. Histologic dating showed variability between patients as well as between different locations within the uterus. When comparing average dating results for each patient, we saw a positive correlation between histologic and ERA dating (Spearman Rho = 0.45); however, this did not reach statistical significance. The ERA results from upper, mid, and lower uterine biopsy specimens were identical for each autologous biopsy, whereas histologic dating showed variability with an average standard deviation of 0.71 days.
Conclusions: The increased heterogeneity of histologic dating is likely to be attributed to the subjectivity of the test. Furthermore, we did not observe a consistent lag or advancement in histologic or ERA dating between the fundal or lower uterine biopsies. Overall, clinicians should be reassured that endometrial tissue will return consistent ERA results independent of the location within the uterus in which it was obtained. (Fertil Steril Rep® 2023;4:375–9. ©2023 by American Society for Reproductive Medicine.)
Key Words: Endometrial receptivity, ERA, histologic dating, Noyes criteria

The luteal phase endometrium has been arguably one of the most studied as well as debated aspects of reproductive endocrinology (1–5). In the first published article in *Fertility and Sterility*, Noyes et al. (6) outlined standard histologic criteria for dating of the luteal phase endometrium. This dating is based on the observation that key events within the glandular and stromal compartments occur within reproducible time periods in the menstrual cycle. Some of these key events include glandular mitosis (occurring early in the cycle), secretory change (occurring midluteal), stromal mitosis, and pseudodecidualization (occurring late) (1, 6). By quantifying each individual criteria within the histologic specimen, an experienced pathologist can deduce the cycle day in which the biopsy was taken with reasonable accuracy (±1 cycle day) (4). As such, asynchrony between histologic dating and the actual dating of an endometrial specimen by >2 days was considered pathologic. This was the basis for a presumed

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"luteal phase defect" and was considered an indication for progesterone support for decades (7).

Today, histologic dating has been largely replaced by expression-based molecular assays (8). The endometrial receptivity array (ERA) is arguably the most widely used of these molecular assays. It is a gene expression-based assay that was built on the presumption that the peak of the window of receptivity occurs 7 days after the luteinizing hormone (LH) surge (LH+7) of a natural cycle (3). Therefore, gene expression signatures from an endometrial biopsy at LH+7 in fertile patients are defined as "receptive." Similarly, gene expression signatures from biopsies outside of the window of receptivity are defined as prereceptive (LH+1 to LH+4) and postreceptive (LH+11 to LH+13) (3). Therefore, the ERA is built on the same premise as histologic dating, but instead of histologic markers, it uses gene expression profiles to date receptivity of the endometrium.

The putative superiority of the ERA over the former method has been attributed to the high degree of subjectivity of human-based observation inherent in histologic dating (4, 9), and the specificity of the ERA gene signature to detect a receptive state (3). Whereas the ERA may be superior in overcoming observer variability, it is not clear if it may be dependent on the region within the uterus from which the specimen was obtained. For example, in their original landmark article, Noyes et al. (6) described the lower uterine segment as unreliable for dating purposes, because of an immaturity of glandular development compared with the fundus. This observation has been echoed widely and mentioned specifically in other works by Mazur and Kurman (10) and Rock and Bartlett (11). If the endometrium is truly heterogeneous from fundus to lower segment, there is potential for varying ERA results depending on the location from which the biopsy is taken.

This problem has direct clinical implications. First, the typical endometrial biopsy is not done under direct observation. Therefore, a clinician might inadvertently sample more tissue from the lower uterine segment compared with the fundus. Second, a single endometrial biopsy may be divided into several portions for different testing (i.e., simultaneous ERA and histologic examination). If an endometrial biopsy is not homogeneous, 2 portions of a divided specimen may yield different results depending on which it is sent for histology or ERA. To address this, we designed a prospective blinded study using a biopsy method designed to maximize the potential for detecting spatial heterogeneity within the endometrium, yet still relevant to the constraints of a typical clinical biopsy.

MATERIALS AND METHODS

Experimental Design

Twelve patients undergoing a mock frozen embryo transfer (FET) cycle with endometrial biopsy as a part of their predetermined clinical plan were recruited to participate in this study. The study protocol was approved by the University of Southern California Institutional Review Board (HS-20-00217). Only patients undergoing biopsy as a part of their clinical care plan were invited to enroll. Three providers were practicing in the clinic at the time of the study. All 3 providers routinely utilized endometrial biopsy (1 utilizing ERA and the other 2 utilizing histologic assessment) for gestational carriers, which represented most participants. Biopsies for other indications were done at the discretion of the physician and/or after specific request for ERA by the patient. All patients underwent a standard FET protocol with oral micronized estradiol 2 mg twice daily for the first 6 days followed by 2 mg 3 times daily for an additional 6–12 days before endometrial thickness check. The endometrial thickness was required to be >7 mm before beginning progesterone. Endometrial biopsy was scheduled after a target period of 108 hours of exogenous progesterone administration, which is the standard protocol for FET at the University of Southern California. All patients administered 50-mg intramuscular (IM) progesterone in the morning and 200-mg vaginal micronized progesterone at midday and evening. Patients were excluded if there was not adequate tissue for all study specimens obtained on one biopsy attempt.

Specimen Collection and Processing

Endometrial biopsies were performed in a systematic manner by first advancing the pipelle (Cooper Surgical Endometrial Sampling Device Pipelle – #8200) to the fundus. Correct fundal depth was confirmed by comparing the biopsy pipelle depth with the depth recorded by prior endometrial sounding at mock transfer. Suction was applied to the pipelle followed by twirling in place until return of tissue was observed in the pipelle beginning at the fundus, miduterus, and lower uterus just distal to the internal ostium before being withdrawn. Collection of tissue at each depth was confirmed visually by directly watching tissue enter the pipelle. Spatial organization of the specimen within the pipelle was preserved on expulsion by cutting the tip of the pipelle so that the tissue was easily expelled into a single line. The biopsy specimen was divided into 3 equal portions. Each of the 3 portions was further divided into an ERA and histology specimen. The tissue sizes for each individual ERA and histology specimen ranged from 3×3 mm to 7×3 mm. If the patient was undergoing biopsy for the purpose of being assessed by ERA, the fundal portion was sent as the clinical specimen, and the patient's chart was accessed at the conclusion of the study to enter the missing ERA result data from the fundal biopsy specimens. For the remaining samples, random numbers were assigned to all specimens, so the study samples were blinded for analysis.

Before agreeing to participate in the study, Igenomix (Valencia, Spain) and the pathologists were made aware of certain information. Igenomix was aware that the purpose of the study was to determine the consistency of ERA results from different portions of the endometrium. Both Igenomix and the pathologist were informed that they would be receiving multiple, randomly numbered endometrial biopsy specimens, some of which are from the same patient. No other further details, of the study were divulged to either party.

Samples were shipped in bulk and randomly ordered so that it would not be possible to determine which specimens originated from the same patient. To ship ERA specimens in bulk, a special protocol for local sample storage had to be

TABLE 1

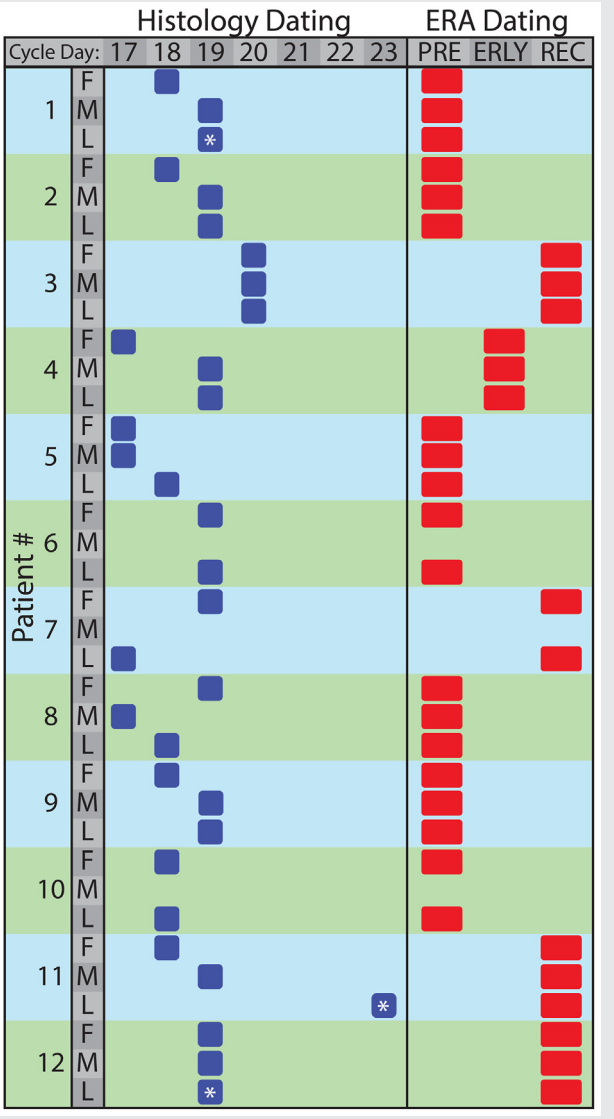
Patient characteristics.

	Mean	SD
Age, y	39.0	6.3
Hours of progesterone	109.7	1.3
Endometrial thickness	9.8	2.5
Diagnosis/indication for mock cycle		
Surrogate/reciprocal IVF	7	
Diminished ovarian reserve	4	
History of leiomyoma	1	

IVF = in vitro fertilization.

Place. ERA vs. histologic dating biopsies. Fertil Steril Rep 2023.

FIGURE 1



Results of histologic and ERA dating. F, M, and L correspond to fundus, miduterus, and lower uterus, respectively. *Pathologist noted insufficient tissue for definitive diagnosis. ERA = endometrial receptivity array.

Place. ERA vs. histologic dating biopsies. Fertil Steril Rep 2023.

devised, otherwise, multiple individual shipments containing 3 samples would have been easily linked to a single patient. Instead, samples were shaken and placed at 4°C for 4 hours then transferred to liquid nitrogen storage until all samples had been collected. Only then were all samples shipped together in the same package. The same cryogenic marker was used to label all of the specimens, and all of the labeling was performed by the same person so that none of the labels had distinctive appearance. To further minimize the potential for data bias, the final ERA results from Igenomix as well as information linking biopsy location to sample codes were first sent to a neutral party. This party subsequently unblinded the data and presented the results to Igenomix and the study team simultaneously.

Histology specimens were processed at the University of Southern California by the Department of Pathology research core. All specimens were processed as they were collected and all slides with tissue sections from the same patient were stained together in the same solution. Once all slides had been processed, they were delivered to the primary investigator and labeled only with a study ID number. They were shipped in bulk to the expert pathologist, who then reported findings back to the primary investigator according to sample ID number.

Statistical Analysis

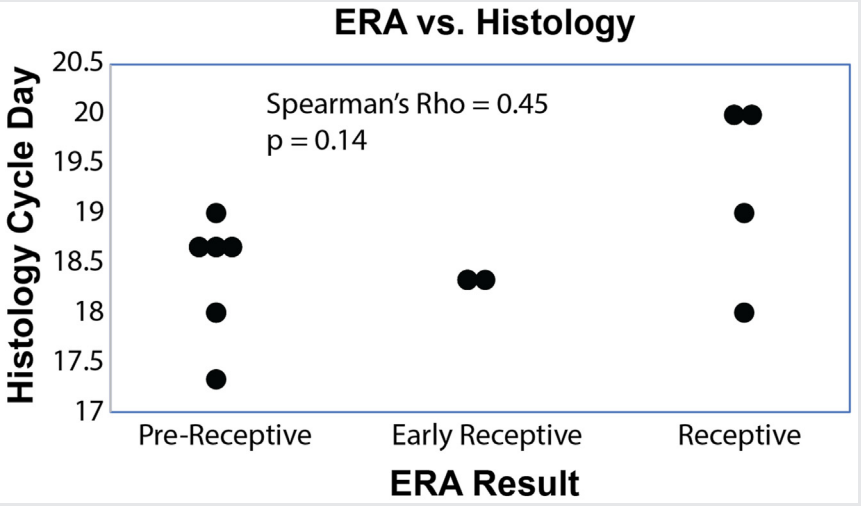
The sample standard deviation (SD) for the 3 histologic biopsies from each patient using the formula $= \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$, with x being the cycle day. Comparison between histologic dating and ERA was made performing spearman ranked correlation. Before ranking, histology dating results were averaged for each patient. The ERA results of "Pre-Receptive," "Early-Receptive," and "Receptive" were given ranks of 1, 2, and 3, respectively. Spearman's Rho was calculated using the means for each autologous set of biopsies using STATA statistical software.

RESULTS

All participants who were enrolled completed the study. Three patients (#6, 7, and 10) did not have adequate tissue to divide into 6 portions, so the specimen was divided into "upper" and "lower" only. The average age of participants enrolled in this study was 39 years (±SD 6.3 years). Mean endometrial thickness was 9.8 mm (±SD 2.5 mm). Most participants (7/12) undergoing endometrial biopsy were surrogates (Table 1).

All specimens submitted for histologic dating were able to be dated, although the pathologist noted scant tissue in 3 of the specimens making dating less certain (Fig. 1). All of the ERA specimens were able to be dated. The sample SD was calculated for each set of biopsies and ranged between 0 and 2.6 days with an average of 0.71 days. Most (92%) autologous biopsies had an SD between 0 and 1.5 days. Specimens were read as corresponding to cycle days 17–23 (day 3–8 postovulation), with 15% day 17, 24% day 18, 48% day 19, 9% day 20 and 3% day 23.

FIGURE 2



Variability in histologic dating. The average dating taken for each autologous histology specimen was calculated and plotted against ERA result. Spearman's Rho was calculated for average histology dating vs. ERA result. ERA= endometrial receptivity array.

Place. ERA vs. histologic dating biopsies. Fertil Steril Rep 2023.

All specimens submitted for ERA returned a result. Of these specimens, 55% were "Pre-Receptive," 10% "Early-Receptive," 35% were "Receptive," and 0 were postreceptive. Postreceptive was not ranked because of no biopsies meeting this criteria. Within each autologous biopsy, ERA results were identical for all specimens, regardless of the location of biopsy (Fig. 1).

When comparing average histologic dating for each patient to ERA, there was a positive correlation between results obtained with the 2 different methods of dating with a Spearman's Rho of 0.45. However, this was not statistically significant (P=.14) (Fig. 2).

DISCUSSION

To our knowledge, this study is the first to directly compare classical histologic dating with molecular dating by ERA on multiple, spatially separated endometrial specimens from the same patient. This study was not meant to evaluate the ability of either histologic dating or ERA to predict the ideal timing of embryo transfer. Rather, we aimed to determine whether purported differences in endometrial receptivity between the fundus and lower uterine segment might be captured within a single endometrial biopsy, and therefore potentially result in confounding ERA results. What we found was that within each patient, endometrial specimens from different locations within the endometrium invariably returned the same ERA result, whereas there was variability in the histologic results. We also found that there was not a consistent histologic pattern of altered maturity in different sections of the uterine fundus. In other words, we did not find that the lower uterine segment lagged behind the fundus or vice versa. Rather, there appeared to be random variability in the histologic readings obtained. It should be noted that the small number of patients in our study is a limitation and may be masking our ability to detect a significant trend.

The variability that we saw in histologic dating between fundal, mid, and lower uterine specimens was very similar to a prior study performed by Noyes in 1956 (9). In that study, Noyes (9) described a series of 100 uteri, each biopsied in 4 distinct locations in anterior and posterior fundus. He found the SD in dating within these autologous sets to range from 0 to 2.1 days, with 95% of sets having an SD between 0 and 1.5 days. In this present study, we found that the sample SD ranged from 0–2.6 days, with 92% of samples falling between 0 and 1.5 days, nearly identical to Noyes's (9) result.

The above study by Noyes (9) was performed on specimens taken specifically from the anterior and posterior fundus, whereas ours were taken in a vertically organized manner from fundus to lower segment, leaving the possibility that variability in our specimens could be attributed to the previously reported differences in maturity between fundal and lower segment endometrium. However, the lower uterine biopsies were not consistently delayed or advanced compared with fundal biopsy specimens. Furthermore, the fact that tissue immediately adjacent to our histologic specimens returned identical results when analyzed with the ERA suggests that the variability seen in histologic dating may be more reflective of the subjectivity of the method than heterogeneity in the tissue itself.

One potential limitation of our study relates to the methodology of biopsy collection and sample division. This method assumes equal representation of each uterine region within a single biopsy sample. Although we realize that an exact 1:1:1 distribution of the biopsy from each region is impractical, we believe that if there were substantial differences in endometrial gene expression between these regions, it would likely manifest as variability in the ERA results

between the submitted sections. An independent validation of this biopsy technique could provide valuable insights into the accuracy of tissue representation from the different uterine areas for future studies wishing to use this technique.

There are several potential explanations for why we were unable to capture significant differences in dating results between the fundus and lower uterine biopsies, despite using a biopsy method intended to maximize the potential for capturing these differences. First, unlike Noyes et al. (9) who visually biopsied hysterectomy specimens, we did not visually biopsy the uterus. Therefore, it is possible that tissue from the true lower uterine segment is lacking in our sample sets. This seems unlikely because we were able to visualize tissue continuing to enter the pipelle when the pipelle was positioned just distal to the internal ostium. The second possibility is that differences in receptivity may not be as prominent in programmed cycles using supraphysiologic hormone levels. Indeed, the original studies were performed on natural cycles. A third possibility is that variability between fundal and lower segment endometrium is not as significant as previously thought, or only occurs in a subset of the population that we did not capture in our small study. Although there are several reports in the literature stating that the lower segment is undatable or unreliably dated compared with the fundus (6, 10, 11), these statements were without reference to peer reviewed studies. In fact, we were unable to locate any peer reviewed studies assessing this phenomenon. Therefore, to definitively answer this question, future studies examining visually directed biopsies of the lower segment and fundus will be required.

Our study does have several additional limitations. This study was limited by the fact that most of the subjects were gestational carriers. It is possible that had we enrolled patients with recurrent implantation failure or luteal phase defect, we may have seen differences in receptivity between different portions of the uterus. The small number of patients in this study and lack of diversity among participants also limits the interpretability and increases risk of bias. However, we believe that the stark contrast between ERA and histologic dating demonstrated by the study is an important contribution to the literature even in the absence of a larger and more diverse participant population.

Another potential source of bias is the fact that Igenomix was aware of the study purpose, whereas the pathologist was not. This additional information was necessary to obtain the participation of Igenomix in the study. It would be potentially harmful to Igenomix for results to show differences in ERA results between autologous endometrial biopsies. Although it is theoretically possible to extract genomic DNA from the samples, sequence it, and use this information to "unblind" the samples, we have no reason to suspect that such an occurrence took place. This is because several of the patients in this study were undergoing endometrial biopsy for the primary purpose of being sent for clinical ERA analysis. These samples were sent to Igenomix well before any study samples were sent. Igenomix was unaware that some of the samples had already been analyzed clinically. Despite this, clinical results returned the same as their autologous study samples in all cases.

CONCLUSION

Overall, we conclude that an endometrial biopsy taken from different positions in the uterus is likely to return variable results when dated histology, whereas the ERA is likely to be consistent. In addition, there is no consistent pattern in the variability seen in histologic samples from different sections of the uterus. These results do not confirm the utility of either of these methods for the evaluation of the endometrium, but do provide important information for the interpretation of the various results.

Declaration of interests: T.L.P. has nothing to disclose. R.A. has nothing to disclose. P.N. has nothing to disclose. S.W. has nothing to disclose. L.K.M. has nothing to disclose. P.K. has nothing to disclose. J.C.F. has nothing to disclose. R.J.P. has nothing to disclose.

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