OPEN ACCESS Freely available online

Journal of Fertilization: In vitro - IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology

Research Article

Ultrasound Based Endometrial Receptivity Scoring Improves *In Vitro* Fertilization Pregnancy Rates

Hannah E. Pierson1, Ken Cadesky[2], Jim Meriano[2], Jesse Invik[1], Carl A. Laskin[2], Roger A. Pierson[1,3]*

[1]Synergyne Imaging Technology, Saskatoon, Saskatchewan, Canada; [2]TRIO Fertility Centre, Toronto, Ontario, Canada; [3]University of Saskatchewan, Saskatoon, Saskatchewan, Canada

ABSTRACT

Background: The endometrium is a key factor in establishment of pregnancy. In IVF therapy, endometrial investigation is generally limited to thickness measurements or occasionally invasive biopsy-based procedures. A non-invasive endometrial receptivity diagnostic (usER test; Matris™, Synergyne Imaging Technologies Inc., Canada.) has recently become available. We performed a retrospective chart review study to test the hypothesis that routine implementation of the ultrasound- based Endometrial Receptivity (usER) diagnostic test would improve pregnancy rates in IVF cycles.

Methods: All patients undergoing IVF at one Canadian reproductive medicine clinic in the 2018 calendar year were considered (n=1521). Patients received either standard of care endometrial thickness and pattern assessments (n=1205) or diagnostic usER testing (n=316) prior to planned embryo transfer. In the usER group, patients with usER scores of 7.0 or above proceeded to embryo transfer (ET; n=246); patients with scores <6.5 (n=70) had their planned embryo transfer deferred and embryos cryopreserved, or retained in cryopreservation, for use in future cycles. Pregnancy (positive beta-hCG) rates were calculated for fresh, frozen-thaw, and aggregate (combined fresh and frozen) ET cycles.

Results: Aggregate pregnancy rates for the usER group were 12% higher than for the Standard of Care group (p=0.0005; 52.0% versus 40.0% respectively). The pregnancy rate for fresh embryo transfer cycles in the usER group was 20.0% higher than that of the Standard of Care group (p=0.0005; 54.9% versus 34.9%, respectively). In frozen embryo transfer cycles, a 9.4% higher pregnancy rate was observed in the usER group than the Standard of Care group (p=0.017; 51.3% versus 41.9% respectively). Implementation of usER resulted in conservation of 64 cryopreserved embryos through deferral of low-probability of pregnancy cycles.

Discussion & Context: This 'real world'/'all patients' retrospective analysis demonstrates that usER testing may be implemented to improve pregnancy rates and conserve embryo potential.

Keywords: Endometrium, Endometrial Receptivity, usER, IVF, pregnancy rate, ultrasound.

INTRODUCTION

Assisted reproduction technologies (ART) have made great advances in increasing the pregnancy rates per embryo transfer in recent decades. Many of the most significant advances have been built upon improving embryo quality by refining laboratory techniques, genetic screening, and increased oocyte quality from optimized ovarian stimulation cycles [1]. Endometrial receptivity is defined as the ability of the uterine lining to accept and provide a suitable environment for a developing embryo. Endometrial receptivity is a critical component in the establishment of pregnancy; however, reliable assessment of uterine receptivity in ART has remained elusive.

The endometrium is under the constant influence of circulating hormones, either endogenous during natural cycles or exogenous during ovarian stimulation when fresh embryo transfer is contemplated. Similarly, cryopreserved embryos may be transferred during a patients' natural cycle or with the use of exogenous hormonal preparation. Reproductively active hormones directly

***Correspondence to:** Roger A. Pierson, Synergyne Imaging Technology, Saskatoon, Saskatchewan, Canada, Tel: +1306-341-3332; E-mail: roger.pierson@usask.ca

Received: October 19, 2021, **Accepted:** November 23, 2021, **Published:** November 30, 2021

Citation: Pierson HE, Cadesky K, Meriano J, Invik J, Laskin CA, Pierson RA (2021) Ultrasound Based Endometrial Receptivity Scoring Improves *In Vitro* Fertilization Pregnancy Rates. J Fertil *In vitro* IVF Worldw Reprod Med Genet Stem Cell Biol 9:6. doi: 10.35248/2375-4508.21.9.248.

influence the physiological changes involved in endometrial tissue proliferation and secretory function. The changes in physiology and accompanying microanatomy become apparent when visualized using ultrasonography as changes in endometrial thickness and pattern. In this regard, endometrial image attributes and their contributions to pregnancy in successful IVF cycles remain underappreciated; there is no consensus on how the images may be interpreted to predict successful implantation. The currently accepted standard for making the decision to proceed to transfer in IVF cycles is ultrasonographic determination of two characteristics: endometrial pattern and thickness. Cycles in which trilaminar endometrial patterns are observed are considered favorable for embryo transfer [2-4]. A previously published study showed that endometrial thickness of less than 7.0 mm was associated with decreased pregnancy rates [5]. Therefore, cycles with endometrial thicknesses less than 7.0 mm are frequently cancelled; however, pregnancies were still established in women that had endometrial thicknesses <7.0 mm [5]. Conditions are considered optimal for embryo transfer when midsagittal endometrial thicknesses are greater than 7.0 mm and exhibit a trilaminar pattern.

At present, reliable standardized evaluation of endometrial receptivity is difficult and the methods developed to do so have generated controversy. Endometrial compaction (decreased thickness between measurements taken at the end of the estrogen phase and the day of embryo transfer) has been proposed as a means of assessing probability of pregnancy [6-8]. Other means of assessment of endometrial receptivity have been limited to invasive biopsy procedures and bench chemistry techniques applied to the biopsy specimens. Receptivity tests such as Endometrial Receptivity Array® (ERA) and Endometrial Receptivity PeakSM (ERPeak) have been suggested as diagnostics that provide insight into the optimal 'implantation window' [9-11]. Clinicians may be advised to adjust the day of embryo transfer based on the determination of the endometrial state being pre-receptive, receptive, post-receptive, or non-receptive. Both tests require patients to undergo a mock IVF cycle. On the last day of the mock cycle, endometrial biopsies are taken instead of performing an embryo transfer (ET). The biopsied tissues are assayed for specific gene markers using high throughput ribonucleic acid (RNA) sequencing methods. An endometrial function test (EFT; not commercially available) was a precursor to the ERA and ERPeak. The EFT test also utilized biopsied tissue from mock cycles to assess levels of a small number of specific proteins associated with endometrial development and for optical histology [12]. The ERA, ERPeak, and EFT methods do not provide a 'real-time' assessment of the endometrium on the cycle for which embryo transfer is being considered. Rather, these techniques rely on tissue collection and assays done one or several months in advance of the cycle in which ET is planned. It is important to consider that interpretation of the tissue assays is based upon the assumption that the cycle in which the tissues are acquired will be representative of every subsequent cycle for that patient. However, significant intercycle variability exists for both medicated and natural cycles for any given woman [13-21]. Reports on the efficacy of the ERA tests are contradictory [22-24].

The cost of generating embryos is significant, both financially and emotionally for patients. Patients often require multiple treatment cycles and rounds of exposure to exogenous gonadotropins. A non-invasive, real-time diagnostic tool to assess uterine receptivity is highly desirable. An 'ultrasound- based Endometrial Receptivity' (usER) test was developed to address this diagnostic gap, provide quantitative per-cycle endometrial receptivity metrics, and is commercially available (Matris™, Synergyne Imaging Technologies Inc., Saskatoon, SK). The usER test is designed to assess the receptivity of the endometrium in each IVF cycle being considered for ET. Echotextural attributes of endometrial tissues were studied using daily ultrasound evaluation of the effects of circulating hormones during natural menstrual cycles, ovulation induction cycles, and fresh and frozen IVF cycles [13;25;26]. Ultrasonographic image processing algorithms were developed to determine the interrelationships among anatomic attributes [13;27-30]. The usER test is based on quantitative image attributes of endometrial tissues reflecting glandular proliferation/differentiation and detailed colorimetric analysis of computer-enhanced 3D surfaces of the endometrial echoes (virtual histology). Recently, we determined that usER scores are strongly correlated with pregnancy outcome and report information fundamentally different than endometrial thickness measurements [31]. The goals of the usER test are: 1) to provide reliable standardization of endometrial criteria for cycle selection; 2) to improve pregnancy rates; 3) to conserve embryo potential; 4) reduce the number of IVF treatment cycles; and, 5) reduce the time required to achieve pregnancy.

To utilize the usER diagnostic, clinicians submit standardized ultrasonographic images of the endometrial tissues (sagittal and transverse) at a pre-determined time in the endometrial preparation protocol. Proprietary image processing and scoring algorithms are used to quantify multiple parameters of endometrial health and receptivity. Physiologic attributes reflected in the ultrasound images are synthesized and condensed into a numeric score representing endometrial receptivity: 0 (poor receptivity) to 10 (optimal receptivity) at 0.5 point intervals. Clinicians are provided a report and endometrial receptivity score within 24 hours of image upload. The usER test was first used in a clinical trial and was efficacious for predicting the probability of pregnancy in women undergoing IVF [25]. Refinements to the analytic process were made in subsequent clinical experience trials prior to the test becoming commercially available. Diagnostic usER provides endometrial receptivity assessment 48 hours prior to each considered embryo transfer thus eliminating the concerns of inter-cycle variation in the endometrial response. Imaging for usER testing is done utilizing ultrasound scanning infrastructure readily available in most IVF clinics. Images are uploaded to a secure central server system over a secure internet connection. Reports are returned to the clinic in under 24 hours. No additional consumables or specialized equipment are required. Diagnostic usER testing provides clinicians with a tool to better understand the endometrial attributes associated with successful IVF outcome and optimize cycle selection. Protocol adjustments to improve endometrial preparation may be made in subsequent cycles when scores are suboptimal [25].

In the context of the present study, cycle selection was considered to mean the clinical decision of whether or not to proceed to embryo transfer following evaluation of the uterine lining. Embryo conservation was understood to mean that embryos not transferred when the usER diagnostic indicated poor endometrial receptivity were deferred for use in future cycles. We expected that selection of cycles with optimal probability of pregnancy would help to mitigate the negative impacts of failed embryo transfers.

The objectives of the present retrospective study were: 1) to determine the effectiveness of implementing a care pathway that includes usER to aid decision making in a clinical setting; and, 2) to test the hypothesis that usER-based cycle selection would improve pregnancy rates and conserve embryo potential.

MATERIALS AND METHODS

All IVF patients (n=1521) from a single tertiary ART clinic who received treatment during the calendar year of 2018 were considered in this retrospective analysis. Nine clinicians provided IVF therapy to patients in 2018 through the clinic. Eight of the physicians were considered to have standard ART practices. One clinician operated a sub-specialty recurrent pregnancy loss practice. One clinician operating a standard ART practice was an early adopter of the technology and implemented diagnostic usER testing for cycle selection (n=316) as routine care for his patients. All of the other 7 physicians (n=1205) operating standard ART practices utilized endometrial thickness and pattern assessments for cycle selection. All patients under the care of the clinicians operating standard ART practices were included in the analysis. Patients under the care of the recurrent pregnancy loss specialist (n=85) were excluded. All patients undergoing infertility therapy at the clinic consented to the use of their data in research and quality assurance analytics as a part of the standard consent for treatment. For the purposes of the present study, pregnancy was defined as positive beta-hCG. The present analysis was conducted in compliance with the tri-council policy statement for ethical conduct for research involving humans TCPS2. Ethical approval was obtained from the University of Saskatchewan Biomedical Research Ethics Board (BIO-2093). The outcome review and comparison of usER to standard of care was conducted retrospectively.

All of the IVF cycles in the analysis had embryos produced in the same laboratory under the direction of a single senior embryologist using standardized technologies. We compared pregnancy outcomes from patients undergoing usER-based cycle selection to patients who received clinical standard of care endometrial thickness and pattern assessments for cycle selection. Data also were partitioned between fresh and frozen cycles in both the usER and standard of care groups for sub-analyses. Finally, we assessed the conservation of embryo potential provided by usER-based cycle selection.

Centralized data handling, analytics, and reporting measures were in place for patients provided with diagnostic usER testing in accordance with standard operating protocols (Synergyne ART Analytics, Saskatoon, SK). Data from usER cycles were jointly held by Synergyne and TRIO Fertility Centre. The seven remaining clinicians used endometrial thickness/pattern assessments as per standard operating procedures at the clinic. Pregnancy outcomes for patients who underwent usER diagnostic testing were calculated and compared to those of patients who received standard of care assessment. Clinical data for all patients were held in the TRIO database. Outcome analyses were conducted jointly by TRIO and Synergyne personnel. Our intent was to provide a straightforward analysis of the impact of diagnostic usER testing when integrated into routine clinical practice with standardized action taken in response to the diagnostic usER score provided.

Study design

This is a retrospective analysis of patients who underwent IVF for infertility treatment using either standard of care endometrial thickness/pattern assessment method (7 clinicians) or the diagnostic usER test (1 clinician). Patients were not assigned to study groups, rather, their care provider determined the use of usER testing. A diagrammatic representation of the study construct is shown (Figure 1).

Patients in the usER group underwent transvaginal ultrasound on day 4 of progesterone support for both fresh and frozen cycles (2 days prior to potential/anticipated day 6 embryo transfer). The endometrial imaging and analysis were not considered to present additional risk to the patient. Mid-sagittal images of the uterine body were obtained as per standard operating procedures at the clinic. Images of the uterus were maximized within the image field of view. The probe was then repositioned to acquire transverse images. Transverse images were acquired at the thickest aspect of the mid-sagittal endometrial echoes of the uterine body, typically between 5 mm and 15 mm from the endometrial-myometrial interface at the fundus. Images were transferred to a secure central server system via internet using a virtual private network and secure clinic portal. Images were analyzed centrally with the usER diagnostic algorithms. Standardized reports providing an endometrial receptivity score were generated for each patient. The usER scores fall onto a 10-point scale; scores with lower numeric values represent low probability of pregnancy and high numeric values represent higher probability of pregnancy (lowest 0, to optimal 10) at 0.5 point intervals. The clinician integrating usER into practice retrieved the reports via a unique log-in to the clinic portal and made the clinical decision to proceed to embryo transfer or to defer embryo transfer to a subsequent cycle with a usER score indicative of a higher probability of pregnancy. Cycles with usER

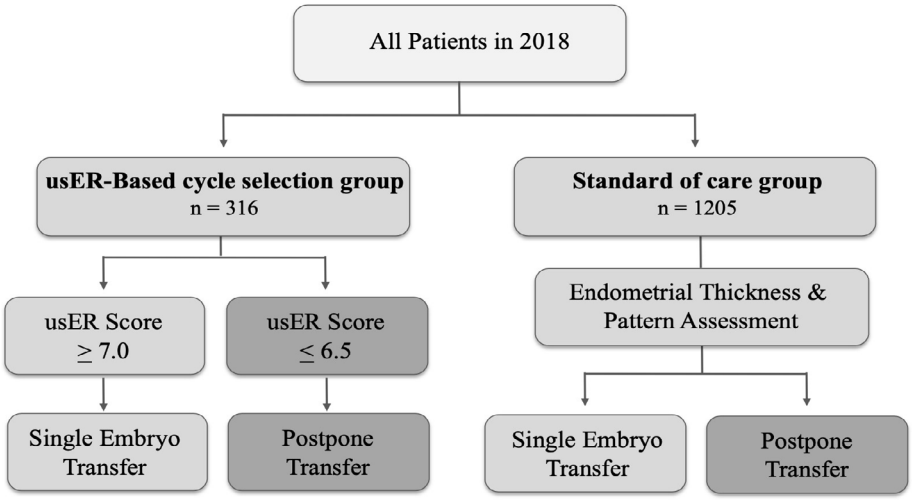

Figure 1: Embryo transfer decision tree in each cycle selection group. Patients undergoing IVF were assigned to either the usER group, or the standard of care group. usER scores of 7.0 or higher received single embryo transfer, while scores at 6.5 or lower did not. Low scoring usER cycles resulted in embryos being cryopreserved for subsequent cycles with optimal usER scores

scores of 7.0 or higher proceeded to embryo transfer based upon a previously determined threshold [25,26]. Women with usER scores <6.5 did not receive embryo transfer. All ET procedures within the usER group were conducted by the same physician. Patients in the standard of care group had routine ultrasound endometrial thickness and pattern assessment for cycle selection following standard operating procedures for the clinic. The standard of care group underwent transvaginal ultrasound at the end of estrogen supplementation/day 1 of progesterone replacement and mid-sagittal endometrial thickness measurements were taken and endometrial pattern assessed. The decision to proceed to embryo transfer was made according to standard operating procedures at the clinic (cycles proceeded to ET if endometrial thickness was 7 mm or greater on day 1 of progesterone supplementation). All embryo transfers were conducted using a standardized clinical embryo transfer tray as per standard operating procedure at the clinic.

At the end of the calendar year the annual pregnancy rate (positive beta-hCG) was determined for the usER and standard of care groups. The difference in annual pregnancy rates between the two groups was calculated. Fresh versus frozen sub-analyses of the pregnancy rates within each group were conducted. All calculations were based on per embryo transfer values. Embryo conservation was calculated by assessment of the number of embryos that were not transferred into sub-optimal endometria (scoring <6.5). Cycles cancelled for other reasons, such as low ovarian response or culture failure was not considered conserved embryos.

Patient demographics

The patients involved in the analysis were not screened based on any selection criteria, thus approximating normal clinical IVF practices. Patient demographic comparisons between the two groups of patients are shown (Table 1 and 3).

Data collection

Diagnostic usER results for each IVF cycle were cross referenced with the clinics database (BabySentry Professional, Medical & Genetics Software Corp., California, USA). The data were accessed using a virtual private network providing end-to-end encryption. Patient information was de-identified for statistical analysis. Data collected were cycle outcome, primary infertility diagnosis, and number of embryos transferred. Outcomes were organized into one of three categories: pregnant (positive pregnancy diagnosis), not pregnant (negative pregnancy diagnosis) and no ET (no embryo transfer performed). Patients for whom there was no recorded outcome in the clinical database (n=15) were eliminated from the analysis. Pregnancy rates and all related data for cycles that did not have usER performed were provided directly by the clinic.

Statistical methods

Analyses were performed using Excel 14.4.1 (Microsoft Corporation, Redmond, Washington, USA) and IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). Percent pregnant per embryo transfer was calculated as: number of cycles with a pregnant outcome/(number of cycles started - cycles with no ET performed) x 100. Chi-square tests were used for categorical variable analyses. Mann-Whitney-U tests were utilized for non-parametric data. The threshold for statistical significance was set at p=0.05.

RESULTS

No differences were observed in percentage of fresh versus frozen transfer cycles, percentage of cycles in which PGS results were known, or percentage of euploid embryos per PGS screened cycle. Patient demographics were similar except that patients in the usER group were younger by a mean difference of 0.8 ± 0.2 years and the primary reason for infertility was higher in the usER group than in the standard of care group ($p<0.001$ and $p<0.0001$, respectively, Table 1).

Annual pregnancy rates per embryo transfer for the usER group and the standard of care group were 52% and 40% per embryo transfer, respectively (Figure 2A and Table 2). The pregnancy rate in usER- based selection cycles was 12.0% higher than the standard of care group (p=0.0005).

Frozen embryo transfers resulted in a pregnancy rate of 41.9% in the standard of care group and 51.3% in the usER group (Table 2 and Figure 2B). The annual pregnancy rate for fresh embryo

Table 1: Patient Demographic Information.

Variable	usER Group (n, %, or mean)	Standard of Care (n, %, or mean)	p-value
Number of frozen cycles (n)	239	896	-
Number of fresh cycles (n)	77	309	-
Number of cycles total (n)	316	1205	-
Number of cycles with PGS results	22	108	0.219
% euploid embryos per PGS cycle	39.2	46.1	0.4
Percent total cycles fresh vs frozen embryo			
Frozen (%)	75.6	74.4	0.64
Fresh (%)	23.4	25.6	
Number of cycles received ET (n)	246	1187	-
Average patient age (years)	35.4 ± 0.21	36.2 ± 0.12	<0.001
Patient age range (years)	26–49	22–46	
Primary Infertility Diagnosis			
Male Factor Infertility (%)	18.6 (%)	20 (%)	
Female Factor Infertility (%)	49.3 (%)	36 (%)	<0.001
Male and Female Factor Infertility (%)	16.4 (%)	19.4 (%)	
Unknown / Idiopathic Infertility (%)	15.5 (%)	24.3 (%)	

Note: PGS was completed on a minority of frozen cycles (no PGS in fresh cycles).

transfers was 34.9% in the standard of care group and 54.9% in the diagnostic usER testing group (Table 2 and Figure 2C). The pregnancy rates in the usER group were 20% (p=0.0005) higher for fresh cycles and 9.4% (p=0.017) higher for frozen cycles.

The usER group consisted of 316 cycles. Sixty-four cycles were deferred due to low usER scores and the embryos were conserved for use in subsequent cycles. Six of the 316 cycles were cancelled due to culture failure. (Figure 2D and Table 2). The standard of care

Table 2: 2018 IVF cycle data summary.

	Cycles Started	Cycles with ET	Pregnancy Rate	Embryos
	n	n (%)	%	Conserved n (%)
usER				
Fresh	77	51 (66.2%)	54.9%	26 (33%)
Frozen	251	207 (82.4%)	51.3%	44 (17.5%)
Combined	316	246 (77.8%)	52.0%	64 (22.1%)*
Standard of Care				
Fresh	309	309 (100%)	34.9%	0 (0%)
Frozen	896	878 (97.9%)	41.9%	18 (2.0%)
Combined	1205	1187 (98.5%)	40.0%	18 (1.5%)

*Six embryos were not viable on the scheduled day of ET and were not transferred as a result; these 6 embryos were not counted as 'conserved'.

Table 3: Female factor infertility diagnosis categories.

Infertility Factor	usER Group (%)	Standard of Care (%)
Advanced Maternal Age	8	12.9
Donor Egg	2.5	1.2
Diminished Ovarian Reserve	10.4	12.3
Endometriosis	5.1	2.9
Idiopathic	31.2	28.8
Male Factor	22	19.1
Ovulation Disorder	5.2	1.2
Polycystic Ovary	7.7	2.2
Donor Sperm	3.8	6.4
Tubal Factor	3.8	3.8
Unknown	0	6.1
Recurrent Fetal Loss	0	1.6
Uterine Factor	0	1.1

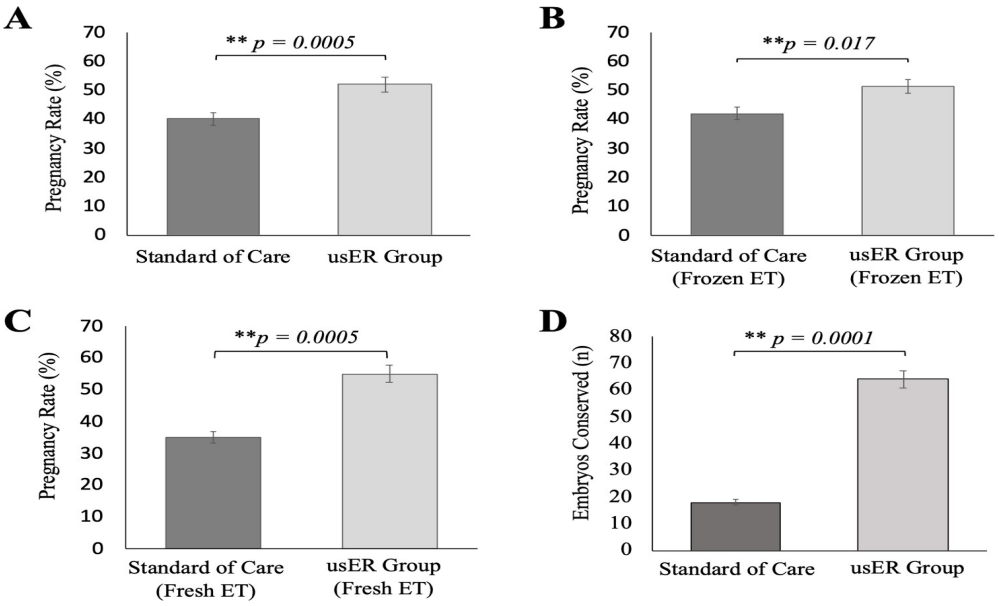

Figure 1: Comparison of pregnancy rates and embryo conservation totals between diagnostic usER- based cycles and standard of care cycles. (A) usER based cycle selection showed a statistically significant 12% higher pregnancy rate than the standard of care group. usER based cycle selection showed statistically significantly higher pregnancy rates in both frozen (B) and fresh embryo transfer (C) categories; 9.4 % and 20.0% respectively. (D) Significantly more embryos were conserved due to cycle deferral in the usER group.

group had a total of 1205 cycles. Eighteen cycles were cancelled. The standard of care group had a lower cycle postponement rate (p=0.0001) resulting in conservation of fewer embryos (n=18).

DISCUSSION

A diagnostic usER test was implemented in a clinical ART practice as an adjunct to clinical decision-making. All patients undergoing IVF at the practice were included in the analysis in order to assess the impact of usER testing in improving pregnancy outcomes. One clinician conducted cycle selection by diagnostic usER and deferred cycles with endometrial receptivity scores less than 7.0. Embryos from deferred cycles were conserved for subsequent cycles with higher probability of pregnancy. Other clinicians providing ART services at the same clinic utilized standard of care endometrial thickness and pattern assessments. The hypothesis that routine implementation of usER testing for cycle selection would improve pregnancy rates was supported. Cycle selection performed using diagnostic usER resulted in a significant increase in annualized pregnancy rate: there was a 20.0% higher annual pregnancy rate observed for fresh cycles and a 9.4% higher pregnancy rate for frozen transfer cycles. Combining fresh and frozen cycle pregnancy rates for patients who had diagnostic usER testing and embryo transfer showed an aggregate pregnancy rate 12.0% higher than patients who received standard of care assessments. Results of the present study are in contrast to a previous report [32].

The use of ultrasonography in assessment of endometrial thickness and pattern is routine in ART practice although the interpretation of the standard assessments and cycle deferrals based on endometrial insufficiency appears to be inconsistent [5]. Standard of care ultrasound assessments are typically performed at the end of estrogen administration or at the beginning of progesterone supplementation [8]. Measurement of endometrial thickness and assessment pattern is quick and non-invasive, making it an attractive option. However, the standard assessments are likely an oversimplified approach to a complex issue. The assessments are easily conducted; however, they lack the sensitivity required for making accurate forecasts of receptivity and assessing the probability of pregnancy. A large cohort study presented findings that higher pregnancy rates were achieved when endometrial thickness was greater than 8 mm [5]. However, the data presented are difficult to interpret. Significantly fewer cycles were observed with each declining millimetre of endometrial thickness, but the reported pregnancy rates among cycles with endometrial thickness <8 mm remained between 24% and 29%. Similarly, the effects on pregnancy rates of endometrial thicknesses that measured greater than 12.0 mm were not addressed; all endometrial thickness measures above 8.0 mm were included in one category. Thresholds for endometrial hyperplasia and other conditions leading to thick endometria were not well defined and appear to have been included in the 'greater than 8.0 mm' cases. Additionally, a study in which over 2200 IVF cycles were analyzed demonstrated that usER scores accurately identified endometria with low probability of supporting pregnancy while endometrial thickness measurements in the same patient population did not [31]. Taken together, there is a growing body of evidence that may be interpreted to mean that the correlation between endometrial thickness and pregnancy outcomes is limited [33,34].

ERA and ERPeak assays were designed to address the problem of oversimplified thickness and pattern assessments by surveying specific gene markers associated with endometrial differentiation to the secretory state. Both methods rely on biopsy of endometrial tissue. Endometrial differentiation from proliferative to secretory phase occurs in a vectoral progression of straight glands developing under the influence of circulating estrogens to becoming branched and coiled beginning at the stratum basalis and progressing toward the lumen as levels of progesterone increase [13]. Given the directional progression in tissue differentiation, the location from which the biopsy is taken may introduce variability into the analysis. Biopsy samples of the endometrium closer to the lumen would be expected result in different microanatomy than a biopsy taken closer to the basalis. A biopsy sampling tissue from the leading edge of the differentiating epithelium also may produce mixed results. Several studies have reported that ERA does not provide improvements in pregnancy rates among typical IVF patients. This observation is consistent with the caveat of variability in tissue properties based on biopsy location. However, patients experiencing recurrent failed embryo transfer do appear to have improved pregnancy rates with the inclusion of ERA [10,35]. Moreover, the biopsy-based methods require lengthy preparation (mock cycles), invasive procedures (biopsies), and do not provide results on a 'per cycle' basis. The assumption that endometrial response in cycles subsequent to the biopsied cycle will be similar is not founded [13-21].

Intracycle variably in endometrial development is significant. The development of analytical tools that may be used to assess endometrial receptivity in each cycle is highly desirable. usER was developed to address this diagnostic gap. A number of image based methods for assessing endometrial receptivity have been identified but have limited predictive utility [36]. Ultrasonographic image attributes have been closely tied to physiological function and hormone-responsive glandular tissue development [13,37-39]. Various approaches to segmentation of the echotextural attributes of hormonally-responsive tissues [28,40-44], correlation of ultrasound image attributes with circulating hormone profiles [45,46], and interpretation of the physiological responses to exogenous hormones [47-50] have been developed using virtual histology techniques [13,27-30]. Taken together, research and clinical data on the usER test support the conclusion that quantitative relationships among various components of the uterine lining and qualitative aspects of 3-dimensional surface models of the endometrium may be used to predict the probability of successful implantation/pregnancy following embryo transfer [25].

The creation of embryos in assisted reproduction has financial and psychological costs for patients and the potential of each embryo has meaning for patients and clinicians: Conserving that potential is a positive proactive practice. Diagnostic usER testing ~48 hours in advance of anticipated embryo transfer provides an opportunity for usER test results to be returned in advance of the typical 'optimal window of implantation'. The usER scores may be used to prevent placement of high-quality embryos into sub- optimal endometrial environments. We observed improved pregnancy rates when embryo transfer was completed in high scoring usER cycles. We also observed conserved embryo potential when embryo transfers were deferred due to low usER scores. Implementation of the usER test in the present study population prevented 64 embryos being placed in what were determined to be suboptimal endometrial environments. Preimplantation genetic screening (PGS) for aneuploidy, in conjunction with ultrasound based endometrial receptivity diagnostic analysis has the potential to improve pregnancy rates even further through pairing of optimal embryos with optimally prepared endometrial environments. In

the present study, PGS was conducted in a minority of cycles that received frozen-thaw ET in both the standard of care and usER groups. PGS was an emerging technology in Canada during the study period and was not routinely utilized. The genetic screening technology was elective and only used at patient discretion. While some improvement in pregnancy rate may be attributed to PGS within both groups, PGS was not conducted on fresh ET cycles in either group. Improvement in pregnancy rate with the usER diagnostic in the fresh ET group was 20% in the absence of PGS testing which was interpreted to mean that usER may be used to improve pregnancy rates as a stand-alone tool.

The clinical decision to proceed to embryo transfer or defer transfer to a future cycle is a multi- factorial process involving logistical constraints, biometrics, clinical experience and foresight, and patient needs. The diagnostic usER test provides a reliable tool for standardized 'present cycle' analytics and supporting rationale to proceed with, or defer, embryo transfer. Deferral of low probability cycles based on diagnostic usER testing results in conservation of embryos. Cryopreserved embryos can be retained for transfer in a future cycle in which endometrial assessment is indicative of a higher probability of successful implantation. Conservation of embryo potential may help patients avoid additional ovarian stimulation cycles and oocyte retrieval procedures, save costs, and may help to prevent the emotionally challenging consequences of failed transfers on patients. Although deferral of an embryo transfer due to sub-optimal endometrial receptivity can be a stressful experience for patients, it has been our experience that adjustment of medicated endometrial preparation protocols can be used to improve usER scores in subsequent cycles. Similarly, in cases where non-medicated cycles are favored, optimal endometrial scores may be observed following those with low scores due to the inherent variability in endometrial response to endogenous hormones.

CONCLUSION

Diagnostic usER testing provides detailed, standardized information on the relative receptivity of the endometrium. The present study provides rationale for standardization of cycle selection based upon usER receptivity scores. Deferral of low probability cycles resulted in significant improvement to pregnancy rates and conservation of embryo potential.

STUDY LIMITATIONS

The present analysis was conducted on a patient population who saw one physician using usER- based cycle selection. The possibility that the improvement in pregnancy rates between the usER group and the standard of care group were related to clinician skill level/transfer efficiency was considered. However, there was a significant difference in the pregnancy rates between fresh and frozen cycles within the usER group. Fresh cycles had nearly 20% higher pregnancy rates than standard of care, while frozen- thaw embryo transfers conducted by the same physician were 10% higher than standard of care. In addition, all embryos were produced in the same laboratory and standardized embryo transfer trays were utilized by all clinicians. If the improvement in pregnancy rates between the usER group and standard of care group were simply related to clinician skill level, one would expect that the improvement in pregnancy rate would be similar among fresh and frozen ET cycles. Future studies with larger patient cohorts and an extended network of clinicians would enhance and expand the predictive value of the diagnostic test. The cycle day on which usER diagnostic (progesterone day 4) and the standard of care group (progesterone day 1) assessments of endometria differed due to the nature of difference in assessment techniques. The patient demographics between the usER group and the standard of care differed slightly; the difference was attributed to the usER group patient population being weighted toward female factor infertility relative to the larger standard of care group. Female factor infertility is considered a more challenging patient cohort and the improvement in pregnancy rate observed in the usER group was not attributed to the infertility type.

ACKNOWLEDGEMENT

We thank, Dr. Kaajal Abrol, Dr. Robert Casper, Dr. Paul Chang, Dr. Beth Gunn, Dr. Michael Hartman, Dr. Dan Nayot, Dr. Sony Sierra, and the staff at the TRIO Fertility Centre for their contributions to data acquisition and data management. We also thank John Deptuch for his assistance in data acquisition and manuscript development. Data analyses were performed collaboratively. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors and was performed using clinic, industry partner and academic resources.

CONTRIBUTOR STATEMENT

HEP co-designed the study and prepared the manuscript. KC co-designed and led the clinical aspects of the study and provided clinical outcome data. JM was lead embryologist, provided data, and conducted statistical analyses for the standard of care patient cohort. JI provided data and conducted statistical analyses on the usER group patient cohort and conducted statistical comparisons between the study populations. CL participated in study development, manuscript development and data analysis.

RAP led the scientific aspects of the study, participated in data collection and analysis, and preparation of the manuscript. All authors have reviewed and approved the manuscript.

DISCLOSURE OF CONFLICT OF INTEREST

RAP is Distinguished Professor of Obstetrics and Gynecology at the University of Saskatchewan and President and CSO at Synergyne ART Technologies. HP and JI are employees of Synergyne ART Analytics. KC and CL are equity and founding partners of TRIO Fertility Centre. KC is the medical director of TRIO Fertility Centre. JM is an employee of TRIO Fertility Centre.

REFERENCES

1. Eskew AM, Jungheim ES. A history of developments to improve in vitro Fertilization. Mo Med. 2017;114(3):156-159.
2. Gonen Y, Casper RF. Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for in vitro fertilization (IVF). Journal of in Vitro Fert Embryo Transf. 1990;7(3):146-152.
3. Heger A, Sator M, Pietrowski D. Endometrial Receptivity and its Predictive Value for IVF/ICSI-Outcome. Geburtshilfe Frauenheilkd. 2012;72(8):710-715.
4. Chen SL, Wu FR, Luo C, Chen X, Shi XY, Zheng HY, et al. Combined analysis of endometrial thickness and pattern in predicting outcome of in vitro fertilization and embryo transfer: A retrospective cohort study. Reprod Biol Endocrinol. 2010;8:30.
5. Liu KE, Hartman M, Hartman A, Luo ZC, Mahutte N. The impact of a thin endometrial lining on fresh and frozen-thaw IVF outcomes: An analysis of over 40 000 embryo transfers. Hum Reprod. 2018;33(10):1883-1888.

6. Haas J, Smith R, Zilberberg E, Nayot D, Meriano J, Barzilay E, et al. Endometrial compaction (decreased thickness) in response to progesterone results in optimal pregnancy outcome in frozen-thawed embryo transfers. Fertil Steril. 2019;112(3):503-509.e1.

7. Riestenberg C, Quinn M, Akopians A, Danzer H, Surrey M, Ghadir S, et al. Endometrial compaction does not predict live birth rate in single euploid frozen embryo transfer cycles. J Assist Reprod Genet. 2021;38(2):407-412.

8. Bu Z, Yang X, Song L, Kang B, Sun Y. The impact of endometrial thickness change after progesterone administration on pregnancy outcome in patients transferred with single frozen-thawed blastocyst. Reprod Biol Endocrinol. 2019;17(1):99.

9. Diaz-Gimeno P, Horcajadas JA, Martinez-Conejero JA, Esteban FJ, Alama P, Pellicer A, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. Fertil Steril. 2011;95(1):50-60.

10. Ruiz-Alonso M, Blesa D, Diaz-Gimeno P, Gomez E, Fernandez-Sanchez M, Carranza F, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril. 2013;100(3):818-824.

11. Enciso M, Carrascosa JP, Sarasa J, Martinez-Ortiz PA, Munne S, Horcajadas JA, et al. Development of a new comprehensive and reliable endometrial receptivity map (ER Map/ER Grade) based on RT-qPCR gene expression analysis. Hum Reprod. 2018;33(2):220-228.

12. Kliman HJ, Honig S, Walls D, Luna M, McSweet JC, Copperman AB. Optimization of endometrial preparation results in a normal endometrial function test (EFT) and good reproductive outcome in donor ovum recipients. J Assist Reprod Genet. 2006;23(7-8):299-303.

13. Baerwald AR, Pierson RA. Endometrial development in association with ovarian follicular waves during the menstrual cycle. Ultrasound Obstet Gynecol. 2004;24(4):453-460.

14. Casper RF. It's time to pay attention to the endometrium. Fertil Steril. 2011;96(3):519-521.

15. Mariee N, Tuckerman E, Ali A, Li W, Laird S, Li TC. The observer and cycle-to-cycle variability in the measurement of uterine natural killer cells by immunohistochemistry. J Reprod Immunol. 2012;95(1-2):93-100.

16. Murray MJ, Meyer WR, Zaino RJ, Lessey BA, Novotny DB, Ireland K, et al. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. Fertil Steril. 2004;81(5):1333-1343.

17. Ordi J, Creus M, Quinto L, Casamitjana R, Cardesa A, Balasch J. Within- subject between-cycle variability of histological dating, alpha v beta 3 integrin expression, and pinopod formation in the human endometrium. J Clin Endocrinol Metab. 2003;88(5):2119-2125.

18. Ojha K, Barnes SC, Boa FG, Moody S, Sladkevicius P, Nargund G, et al. Intraindividual hormonal variability in ultrasonographically timed successive ovulatory menstrual cycles is detected only in the luteal phase in infertility patients. J Assist Reprod Genet. 2002;19(8):363-367.

19. Rossavik IK, Gibbons WE. Variability of ovarian follicular growth in natural menstrual cycles. Fertil Steril. 1985;44(2):195-199.

20. Lenton EA, Landgren BM, Sexton L. Normal variation in the length of the luteal phase of the menstrual cycle: Identification of the short luteal phase. Br J Obstet Gynaecol. 1984; 91(7): 685-689.

21. Fehring RJ, Schneider M, Raviele K. Variability in the phases of the menstrual cycle. J Obstet Gynecol Neonatal Nurs. 2006;35(3):376-384.

22. Bassil R, Casper R, Samara N, Hsieh TB, Barzilay E, Orvieto R, et al. Does the endometrial receptivity array really provide personalized embryo transfer? J Assist Reprod Genet. 2018;35(7):1301-1305.

23. Tan J, Kan A, Hitkari J, Taylor B, Tallon N, Warraich G, et al. The role of the endometrial receptivity array (ERA) in patients who have failed euploid embryo transfers. J Assist Reprod Genet. 2018;35(4):683-692.

24. Shi C, Han HJ, Fan LJ, Guan J, Zheng XB, Chen X, et al. Diverse endometrial mRNA signatures during the window of implantation in patients with repeated implantation failure. Hum Fertil (Camb). 2018;21(3):183-194.

25. Pierson R, Mrazek M, Kuzcynskiw W, Klein BM, Arce JC. Endometrial quality at the end of controlled ovarian stimulation predicts ongoing pregnancy rate after transfer of a single expanded or hatching/hatched blastocyst on day 5 in a fresh cycle. Fertil Steril. 2012;98(3):S225.

26. Cadesky KPH, Laskin CA, Meriano J, Invik J, Pierson RA. Ultrasound Image-Based Scoring System Improves IVF Pregnancy Rates. Proceedings of the Annual Conference of the Canadian Fertility and Andrology Society. 121.

27. Gupta S, Chauhan RC, Sexana, SC. Wavelet-based statistical approach for speckle reduction in medical ultrasound images. Med Biol Eng Comput. 2004;42:189-192.

28. Eramian MG, Adams GP, Pierson RA. Enhancing ultrasound texture differences for developing an in vivo 'virtual histology' approach to bovine ovarian imaging. Reprod Fertil Dev. 2007;19(8):910-924.

29. HaraLick RM, Shanmugam K, Dinstein Ih. Textural features for image classification. IEEE Trans Sys Man Sybern. 1973;3(6):610-621.

30. Singh J, Pierson RA, Adams GP. Ultrasound image attributes of the bovine corpus luteum: Structural and functional correlates. J Reprod Fertil. 1997;109(1):35-44.

31. Pierson HE, Cadesky K, Meriano J, Invik J, Laskin CA, Pierson RA. Ultrasound based endometrial receptivity scoring accurately identifies IVF cycles with low probability of pregnancy. Fertil Steril. 2021; 116(3):E-312.

32. Samara N, Casper RF, Bassil R, Shere M, Barzilay E, Orvieto R, et al. Sub-endometrial contractility or computer-enhanced 3-D modeling scoring of the endometrium before embryo transfer: Are they better than measuring endometrial thickness? J Assist Reprod Genet. 2019;36(1):139-143.

33. Kasius A, Smit JG, Torrance HL, Eijkemans MJC, Mol BW, Opmeer BC, et al. Endometrial thickness and pregnancy rates after IVF: A systematic review and meta-analysis. Hum Reprod Update. 2014;20(4):530-541.

34. Groenewoud ER, Cohlen BJ, Al-Oraiby A, Brinkhuis EA, Broekmans FJM, de Bruin JP, et al. Influence of endometrial thickness on pregnancy rates in modified natural cycle frozen-thawed embryo transfer. Acta Obstet Gynecol Scand. 2018;97(7):808-815.

35. Hashimoto T, Koizumi M, Doshida M, Toya M, Sagara E, Oka N, et al. Efficacy of the endometrial receptivity array for repeated implantation failure in Japan: A retrospective, two-centers study. Reprod Med Biol. 2017;16(3):290-296.

36. Pierson RA. Imaging the endometrium: Are there predictors of uterine receptivity? J Obstet Gynaecol Can. 2003;25(5):360-368.

37. Baerwald AR, Adams GP, Pierson RA. Characterization of ovarian follicular wave dynamics in women. Biol Reprod. 2003;69(3):1023-1031.

38. Eden JA, Place J, Carter GD, Jones J, Alaghband-Zadeh, J, Pawson ME. What are the ultrasound and biochemical features of impending ovulation? Aust N Z J Obstet Gynaecol. 1988;28(3):225-227.

39. Katayama T. Ultrasonographic changes in the endometrium during ovulatory cycles-correlation to serum estradiol and progesterone concentrations. Nihon Sanka Fujinka Gakkai Zasshi. 1990;42(11):1530-1536.

40. Muzzolini R, Yang YH, Pierson R. Multiresolution texture segmentation with application to diagnostic ultrasound images. IEEE Trans Med Imaging. 1993;12(1):108-123.

41. Pierson RA, Adams GP. Remote assessment of ovarian response and follicular status using visual analysis of ultrasound images. Theriogenology. 1999;51(1):47-57.

42. Rusnell BJ, Pierson RA, Singh J, Adams GP, Eramian MG. Level set segmentation of bovine corpora lutea in ex situ ovarian ultrasound images. Reprod Biol Endocrinol. 2008;6:33.

43. Dong M, Eramian MG, Ludwig SA, Pierson RA. Automatic detection and segmentation of bovine corpora lutea in ultrasonographic ovarian images using genetic programming and rotation invariant local binary patterns. Med Biol Eng Comput. 2013;51(4):405-416.

44. Bian N, Eramian MG, Pierson RA. Evaluation of texture features for analysis of ovarian follicular development. Med Image Comput Comput Assist Interv. 2006;9:93-100.

45. Duggavathi R, Bartlewski PM, Pierson, RA, Rawlings NC. Luteogenesis in cyclic ewes: Echotextural, histological, and functional correlates. Biol Reprod. 2003;69(2):634-639.

46. Townson DH, Pierson RA, Ginther OJ. Characterization of plasma progesterone concentrations for two distinct luteal morphologies in mares. Theriogenology. 1989;32(2):197-204.

47. Birtch RL, Baerwald AR, Olatunbosun OA, Pierson RA. Ultrasound image attributes of human ovarian dominant follicles during natural and oral contraceptive cycles. Reprod Biol Endocrinol. 2005;3(12).

48. Pierson RA, Archer DF, Moreau M, Shangold GA, Fisher AC, Creasy GW. Ortho Evra/Evra versus oral contraceptives: Follicular development and ovulation in normal cycles and after an intentional dosing error. Fertil Steril. 2003;80(1):34-42.

49. Baerwald AR, Olatunbosun OA, Pierson RA. Effects of oral contraceptives administered at defined stages of ovarian follicular development. Fertil Steril. 2006;86(1):27-35.

50. Malhi PS, Adams GP, Pierson RA, Singh J. Bovine model of reproductive aging: Response to ovarian synchronization and superstimulation. Theriogenology. 2006;66(5):1257-1266.