Presence of endometrial nucleolar channel systems at the time of frozen embryo transfer in hormone replacement cycles with successful implantation

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Objective: To detect nucleolar channel systems (NCSs) in cells in endometrial aspirations obtained immediately before embryo transfer during blastocyst hormone replacement therapy-frozen embryo transfer (HRT-FET) cycles without affecting implantation.

Design: Prospective case series.

Setting: University-affiliated fertility clinic.

Patient(s): Five patients who underwent an HRT-FET cycle consented to lower uterine segment aspiration using an open-tip embryo transfer catheter during a routine mock transfer performed immediately before embryo transfer.

Intervention(s): Exfoliated cells in the aspirated endometrial secretions were analyzed for the presence of NCSs using indirect immunofluorescence and, in one case, electron microscopy for unambiguous identification.

Main Outcome Measure(s): On the basis of a previous study, positive NCS status was defined as the presence of NCSs in at least 3 endometrial epithelial cells (EECs). The effect of endometrial aspiration on implantation and pregnancy outcomes was assessed.

Result(s): Biochemical pregnancy, as evidenced by positive β-human chorionic gonadotropin, was seen in 5 of 5 patients, and clinical pregnancy was seen in 2 of 5 patients. NCSs were detected in exfoliated EECs of uterine secretions in 4 of 5 patient samples and could not be unequivocally identified in 1 of 5 patient samples, which was designated as indeterminate.

Conclusion(s): This is the first report of NCS detection in HRT-FET cycles in the absence of follicular development and ovulation. NCS status can be determined in exfoliated EECs of uterine secretions obtained at the time of embryo transfer while maintaining implantation. Our study furthers the goal of establishing whether individualized point of care testing of NCS status in HRT-FET cycles can determine optimal endometrial receptivity and improve pregnancy outcomes. (Fertil Steril Sci® 2021;2:80–7. ©2021 by American Society for Reproductive Medicine.)

Key Words: Endometrial receptivity, frozen embryo transfer, nucleolar channel systems, window of implantation

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Successful implantation in assisted reproductive technology relies on the cross-talk between a competent embryo and a receptive endometrium. Significant research has gone into optimizing embryo selection to improve the live birth rate (LBR) with techniques such as extended embryo culture with morphologic grading, time lapse imaging, and preimplantation genetic testing (1). However, further improvement in LBR through the optimization of embryo transfer (ET) timing has remained challenging. We know that transferring embryos into a dysynchronous endometrium decreases the implantation rate and increases the miscarriage rate, and it has
been well established that controlled ovarian hyperstimulation (COH) used in fresh in vitro fertilization-ET cycles can shift the window of implantation (2–6). In frozen embryo transfer (FET) cycles, hormonal control of the endometrium using hormone replacement therapy (HRT) is specifically optimized for implantation and embryo–endometrial asynchrony caused by COH can be avoided (7). With FET cycles now outnumbering fresh ET cycles, identifying the optimal window of implantation to improve the timing of FETs remains critical (8).

Nucleolar channel systems (NCSs) were discovered with the advent of electron microscopy in cell biology. These micron-sized intranuclear organelles, consisting of several layers of membrane tubules embedded in an electron-dense matrix, are specific to healthy human endometrial epithelial cells (EECs) of the mid-luteal phase (9–14). Although their function remains unknown, NCSs have withstood the test of time as bona fide markers of the midluteal WOI. Their presence is independent of fertility status; nevertheless, NCSs are most likely prerequisites of a receptive endometrium (14). NCSs are uniformly distributed throughout the upper uterine cavity as opposed to the lower uterine segment, where there is a reduced likelihood of implantation (15). The sensitivity of NCS timing relative to endometrial maturation is highlighted during COH when NCSs form prematurely (6). Moreover, NCS formation is dependent on a serum progesterone threshold consistent with ovulation (16). Finally, in natural menstrual cycles, the prevalence of NCSs mirrors a transcriptomic signature called the endometrial receptivity array (ERA) that phases the endometrium into proliferative, prereceptive, receptive, and postreceptive periods (17–19).

Transcriptomic assays such as the ERA are commercially available for personalized FET timing in patients with a history of implantation failure, but the ERA has yet to show improvement in LBR, which brings its utility into question (20–22). When applied at the first appointment, however, a recent randomized, controlled trial using the ERA as a personalized guide for ET showed significant improvement in pregnancy, implantation, and cumulative LBRs (23). The ERA requires a mock FET cycle in which HRT is given in the same regimen as was done in a prior failed FET. An endometrial biopsy is performed on the day of planned ET, after which the endometrium is no longer suitable for implantation in that cycle (20). This means that an additional FET cycle is required, with adjustment in the timing of ET on the basis of the results of ERA. There are clear downsides to this method in that there is no guarantee that the subsequent transfer cycle will act the same as the mock transfer cycle. Additionally, this method wastes valuable time and money because of the need for a mock cycle. Ideally, an endometrial receptivity test would be done as a point of care test providing a same-day result and with minimal endometrial disruption, allowing for transfer in the same day or cycle. The most promising approach for this is uterine secretion aspiration (24–28). Indeed, uterine secretion aspiration is compatible with same-day ET, and the secretions contain a significant number of exfoliated EECs (24). To date, a transcriptome-based assay using uterine secretion aspiration has not become available for clinical use. Such tests could run into problems because variable numbers of cells are present in the aspirate, including stromal cells and EECs, at unknown ratios that affect the outcome and reproducibility.

NCS detection could be a viable alternative for assessing endometrial receptivity, with clear benefits over alternative methods. We have previously established that the presence of NCSs can be reliably detected in exfoliated EECs of uterine secretions, with a significant correlation with NCS prevalence in simultaneously obtained biopsies (29). With this method, we know clearly that EECs are present, since they are visualized directly under a microscope. This allows us to establish whether the sample is analyzable, whereas with transcriptomic analysis, mRNA from different cell types is combined into 1 sample, without the possibility of identifying which cell types are present. To expand this hypothesis, we need to study the association between the presence of NCSs and pregnancy outcomes in hormone replacement therapy-frozen embryo transfer (HRT–FET) cycles, which are used most commonly in patients with implantation failure (7, 22). Until now, NCSs have been studied extensively in natural ovulatory cycles as well as in COH cycles where superovulation is present. NCSs have not yet been identified in FET cycles using hormone replacement regimens to prepare the endometrium for ET.

The objective of this study was to determine whether NCSs are present in HRT–FET cycles at the time of ET while achieving successful implantation. We hypothesized that NCSs can be detected as a marker of the WOI in exfoliated EECs of uterine secretions at the time of ET, despite the absence of follicular development and ovulation.

### MATERIALS AND METHODS

#### Subjects

The Albert Einstein College of Medicine/Montefiore Medical Center institutional review board approved this study, and all patients consented to the study protocol. Participants were required to be between 18 and 45 years of age and undergoing a blastocyst HRT–FET as a part of their treatment plan. On the day of ET, our usual protocol was to perform a “mock” transfer. A mock transfer entails introducing a closed-tip catheter through the cervix under ultrasound guidance until the endometrial cavity is entered to a distance of 1–2 cm. The purpose was to evaluate the shape of the cervical canal in preparation for the real transfer. For the mock transfer, we used an open-tip catheter (Wallace Sureview, Origio, Malov, Denmark) attached to a 1-mL syringe, and once the endometrial cavity was entered, the catheter was positioned in the lower uterine segment and the plunger was pulled back by 0.5 mL, creating a small amount of suction that was enough to obtain secretions but not enough to cause damage to the endometrium. Aspiration was only performed if the position of the catheter was in the lower uterine segment and could be visually confirmed by ultrasound. This was to ensure avoiding the fundus and upper uterine segment where the embryo was most likely to implant. There was no blood noted on any of the mock transfer catheters. The secretions...
were immediately transferred into 300-µL phosphate-buffered saline for transport. In 2 cases, sloughed-off clusters of coherent cells were noted within the secretions. These clusters were fixed with 4% paraformaldehyde for 4 hours at room temperature and then kept at 4°C until paraffin embedding.

**HRT-FET Protocol**

All patients underwent a nonpreimplantation genetic testing blastocyst HRT-FET cycle. They were started on estrogen therapy on the third day of spontaneous menses or after undergoing Lupron down-regulation, depending on their clinical history and physician preference. All participants were started on estrogen replacement for at least 2–3 weeks, with a goal estradiol level of 200–500 pg/mL and an endometrial thickness of at least 7 mm. The ovaries were assessed for the presence of a dominant follicle at the time of ultrasound evaluation of endometrial stripe. If a dominant follicle was identified, the serum levels of progesterone and luteinizing hormone were measured before starting progesterone therapy to rule out breakthrough ovulation.

Progesterone therapy was then started, with an ET performed on the 7th day of therapy. We aimed for a serum progesterone level of 15–20 ng/mL around the time of ET. In addition, all patients received 4 days of doxycycline (100 mg) twice a day and methylprednisolone (16 mg) daily starting the day after the initiation of progesterone therapy. Hormone replacement was continued through the pregnancy test 9 days after ET. A positive pregnancy test was defined as a serum β-human chorionic gonadotropin level of >5 U/mL. If the test was positive, hormone replacement was continued until about 12 weeks of estimated gestational age or until a pregnancy loss was diagnosed. Pregnancy was defined as the presence of a positive pregnancy test, clinical pregnancy was defined by the presence of a pregnancy sac on ultrasound, and live birth was defined as delivery of an infant at an estimated gestational age of ≥24 weeks.

**NCS Detection in Uterine Secretions**

NCSs in cells within endometrial fluid were detected as previously described (29). Briefly, sloughed-off EECs in uterine secretions were collected in 300-ml phosphate-buffered saline and sedimented onto slides using a StatSpin CytoFuge 2 (Beckman Coulter, Inc., Brea, CA) at 1,000 rpm for 5 minutes. The cells were fixed at room temperature with 4% paraformaldehyde for 15 minutes and permeabilized with 1% Triton X–100 for 5 minutes. After blocking with 1% dried milk in phosphate-buffered saline for 15 minutes, EECs were stained with rabbit antibodies against cytokeratin 8 (diluted 1:200; Thermo Scientific/Lab Vision, Fremont, CA), NCSs and nuclear pore complexes were stained with mouse monoclonal antibody (mAb414 diluted 1:5,000; Covance Research Products, Princeton, NJ) against a subset of nuclear pore complex proteins that are enriched in NCSs. Fluorescently labeled secondary antibodies (Jackson Immunoresearch, West Grove, PA) were used to detect the rabbit antibodies (rhodamine-labeled goat anti-rabbit at 1:200) and mouse antibodies (DyLight488 goat anti-mouse at 1:500). Primary antibodies were incubated for at least 60 minutes, and secondary antibodies were incubated for at least 30 minutes in blocking buffer at room temperature. Nuclei were counterstained with the DNA stain 4',6-diamidino-2-phenylindole (Sigma, St. Louis, MO). Paraffin-embedded cell clusters were deparaffinized and stained as described in our previous publications (6, 12, 15).

**Light Microscopy**

Slides with cells from uterine secretions were imaged with a Zeiss Axio Observer CLEM (correlative light and electron microscopy) microscope using a 63x/1.4 NA objective equipped with a Zeiss AxioCam HRc camera to acquire Z-stacks (Zeiss, White Plains, NY). The slides were manually scanned until all EECs from uterine secretions were evaluated (29). The prevalence of NCS in paraffin-embedded cell clusters was determined with the use of Z-stacks of endometrial epithelial glands (15).

**Electron Microscopy**

Loose cells in uterine secretions were fixed with 4.0% glutaraldehyde in 0.1 M sodium cacodylate, enrobed in 6% gelatin, and then refixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate. The biopsy specimen was fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate. Both sample types were postfixed with 1% osmium tetroxide followed by 2% uranyl acetate, dehydrated through a graded series of ethanol, and embedded in LX112 resin (LADD Research Industries, Williston, VT). Ultrathin sections were cut on a Leica Ultracut UC7, stained with uranyl acetate followed by lead citrate, and viewed on a JEOL 1200EX transmission electron microscope at 120 kV.

**NCS Analysis**

Uterine secretions contain a multitude of different cell types, especially different types of blood cells, endometrial epithelial and stromal cells, and, in some cases, copious sperm cells. EECs were identified by cytokeratin staining and prominent signal from mAb414 labeling of NCSs and nuclear pore complexes. In fact, the mere presence of NCSs identifies EECs, as NCSs are absent from any other cell type or tissue (12). Because of the variable numbers of cells collected in uterine secretions, samples were assigned to 1 of 3 categories: NCS positive, NCS negative, and indeterminate. Samples containing at least 3 NCSs were given a status of NCS positive. Samples containing at least 30 EECs but no NCSs were designated NCS negative. Samples containing <30 EECs and <3 NCSs were designated indeterminate. This categorization was established in a prior study comparing the presence of NCS in uterine secretions vs. endometrial biopsy specimens (29). In the paraffin-embedded cell clusters, all glands were imaged with the use of Z-stacks, and NCS prevalence was noted in each gland by counting the total number of NCSs present per the total number of EECs (15).
RESULTS

Five patients underwent lower uterine segment aspiration at the time of a mock transfer performed immediately before FET. Demographic information, including age, body mass index, infertility diagnosis, gravidity, parity, and serum anti-müllerian hormone (AMH) levels, is shown in Table 1. The patients’ ages ranged from 33 to 42 years, and they were all either overweight or obese. The most common diagnosis was tubal factor infertility, although 2 patients also had uterine factor infertility due to Asherman’s syndrome. While all patients had prior pregnancy losses, only 1 patient had a prior live birth. The patients had normal ovarian reserve, as evidenced by AMH > 1 ng/mL in all cases. Patient 2 had breakthrough follicular development noted on ultrasound while receiving estrogen therapy, but her progesterone level remained low before starting exogenous progesterone, and there was no sign of ovulation. Although unlikely, the possibility that this follicle contributed to endogenous progesterone cannot be ruled out. The remaining patients had no follicular development noted on ultrasound before the start of exogenous progesterone. Therefore, spontaneous ovulation was generally ruled out. The pregnancy rate was noted to be 5 out of 5, as evidenced by a positive pregnancy test. From there, 2 of 5 patients went on to have clinical pregnancies that ended as spontaneous abortions. Of the 2 patients with spontaneous abortions, 1 had a twin anembryonic pregnancy at the age of 34 years and the other had a singleton anembryonic pregnancy at the age of 42 years. Both opted for the medical management of their missed abortion, and cytogenticics of the products of conception were not obtained. There were no live births in the study group.

After previously characterizing NCSs in EECs of uterine secretions by light microscopy (29), we intended to unambiguously identify them on the ultrastructural level. For this purpose, an endometrial biopsy sample and a uterine secretion sample, which were obtained simultaneously from one of the patients in our prior study (29), were visualized by transmission electron microscopy. An NCS in a glandular EEC was readily identified in the biopsy specimens (Fig. 1A, a). The NCS showed the hallmark triad of membrane tubules embedded in an electron-dense matrix surrounding an amorphous core (Fig. 1A, a’). The small number of cells in the secretions rendered the search for NCSs more challenging, but 1 NCS was unambiguously identified (Fig. 1B). The exfoliation of this cell caused it to show signs of degradation. In particular, the vestigations in the core of and around the NCS were signs of loss of viability (Fig. 1B, asterisks). Despite the partial loss of cytoplasm from the exfoliated cell, the NCS was still clearly visible in the nucleus and shared the hallmark triad with that in the matched biopsy specimen (Fig. 1A, a’). This evidence, together with that from our prior studies (12, 14, 15, 29, 30), demonstrates that the NCSs detected by light microscopy in uterine secretion cells were bona fide NCSs (Fig. 1C, c).

Of the 5 patients, 3 were noted to be NCS positive on the basis of the analysis of exfoliated EECs sedimented from uterine secretions (Table 2). As seen in Figure 1C, the EECs were identified through cytokeratin staining (c’), and NCSs were pinpointed within the 4’,6-diamidino-2-phenylindole-stained nucleus (c”) through mAb414 labeling (c, arrows). The remaining 2 samples were deemed indeterminate for NCS status on the basis of the analysis of uterine secretions alone due to the insufficient numbers of EECs (< 30) to obtain a negative NCS result. In 2 of 5 patients, fragments of coherent cell clusters were identified within the aspirate, which were fixed and paraffin embedded. These were also assessed for the presence of NCS (Fig. 1D). The paraffin-embedded cell clusters each contained 4 glands, which fall short of the 10 glands necessary to reliably calculate the prevalence of NCS representative of the entire endometrium (15). However, on the basis of the available data, the average prevalence of NCSs per gland in patient 1 was 26.1% and ranged from 0% to 41.5% for the individual glands (Table 3). In patient 5, the average prevalence of NCS per gland was 40.6% and ranged from 35.4% to 44.1% for the individual glands. Altogether, 4 of 5 patients had NCSs identified in either isolated EECs (Fig. 1C), paraffin-embedded EECs (Fig. 1D), or both. Table 3 provides a detailed description of the HRT-FET cycles in which cells from uterine secretions were analyzed for the presence of NCSs using indirect immunofluorescence microscopy. No difference in NCSs was noted between the small miscarriage and no-miscarriage groups. Similarly, the small sample size did not allow the presence of NCS to be correlated with pregnancy outcomes. In addition, 2 of the patients had severe Asherman’s syndrome, with fluid in the cavity noted during estrogen stimulation. One of these patients underwent endometrial aspiration of this intracavitary fluid before starting progesterone, and other patient had the resolution of the fluid after the commencement of progesterone. Both of these patients had a biochemical pregnancy after ET.

DISCUSSION

This is the first study to establish the presence of NCSs, a well-recognized marker of the WOI, in HRT-FET cycles using endometrial fluid aspiration immediately before ET. Endometrial

| Patient No. | Age (y) | BMI (kg/m²) | Diagnosis | Gravity | Parity | AMH | Embryos transferred | Pregnancy outcome |
|-------------|---------|-------------|-----------|---------|--------|-----|---------------------|-------------------|
| 1           | 33      | 30          | tubal factor, uterine factor | 2       | 0      | 2.51 | 5AA, 5AB            | biochemical       |
| 2           | 37      | 26          | tubal factor | 2       | 0      | 1.43 | 5AA, 4AB            | biochemical       |
| 3           | 40      | 30          | male factor, uterine factor | 1       | 0      | 1.61 | 3BB                 | biochemical       |
| 4           | 34      | 43          | tubal factor | 2       | 0      | 1.70 | 5AB, 4AB            | twin spontaneous abortion |
| 5           | 42      | 42          | unexplained | 4       | 1      | 2.34 | 4AB, 4AB, 3AB       | spontaneous abortion |

Gerber. Endometrial status at embryo transfer. Fertil Steril Sci 2021.
aspiration of uterine secretions is considered a minimally invasive technique, as opposed to endometrial biopsy, and has proven to be compatible with same-day ET without affecting the implantation and pregnancy rates (24). In 2003, endometrial aspiration before ET was first studied in 66 women and showed no significant effect on ongoing pregnancy rates compared to that in controls (24). Subsequent studies including >400 women undergoing endometrial aspiration before ET have shown no decrease in ongoing pregnancy rates compared with that in controls (27, 28). Since the establishment of this technique as compatible with same-day transfer, numerous studies have analyzed various aspects

Transmission electron (A and B) and indirect immunofluorescence (C and D) micrographs of an endometrial biopsy specimen (A) and of cells from uterine secretions (B to D). Transmission electron micrograph of a human endometrial epithelial cell (EEC) with a nucleolar channel system (NCS) (A left panel: boxed and right panel: enlarged) in a biopsy specimen (A) and of a nucleus with an NCS in uterine secretion aspirated simultaneously from the same patient (B). Note the similarities between the NCSs, despite the loss of cytoplasm and the beginning of degradation (vesiculation around the NCS and in its core, asterisks) in (B). Magnifications are indicated. For indirect immunofluorescence of NCSs, cells were stained with monoclonal antibody 414 (mAb414) against a subset of nuclear pore complex (NPC) proteins that are enriched in NCSs (C and D first panels from left: green). In each panel, 2 NCSs are pointed out (arrows), and NPCs in the nuclear envelope of 1 nucleus are highlighted (arrowheads). EECs are identified by staining with cytokeratin antibodies (C and D second panels from left: red), which do not stain the stromal cells (D second and third panels: stars). All cells were counterstained for DNA to identify nuclei (C and D third panels from left: blue). Merged images are shown (C and D last panels from left). Images in C and D are at the same magnification (scale bar in C last panel from left = 10 µm).

Gerber. Endometrial status at embryo transfer. Fertil Steril 2021.
TABLE 2

| Patient | Estrogen dose/route | Endometrial status | NCS | Comments |
|---------|---------------------|--------------------|-----|----------|
| 1       | estradiol valerate 4 mg BIW | Asherman’s syndrome, no fluid | positive | estradiol valerate 2 mg daily |
| 2       | estradiol valerate 2 mg BW | Asherman’s syndrome | positive | subQ |
| 3       | estradiol valerate 2 mg BW | Asherman’s syndrome | positive | PO |
| 4       | estradiol valerate 2 mg BW | Asherman’s syndrome | positive | PO |
| 5       | estradiol valerate 2 mg BW | Asherman’s syndrome | positive | PO |

Note: NCS = Nucleolar channel system. Biweekly: IM = intramuscular; PO = per os; P4 = progesterone; subQ = subcutaneous; TL = trilaminar; PO = per os; P4 = progesterone; subQ = subcutaneous; TL = trilaminar.

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TABLE 3

| Patient | EECs | NCSs | % NCS |
|---------|------|------|-------|
| Gland 1 | 65   | 27   | 41.5  |
| Gland 2 | 68   | 28   | 41.2  |
| Gland 3 | 78   | 0    | 0.0   |
| Gland 4 | 115  | 30   | 26.2  |
| Sum     | 326  | 85   | 26.1  |

Note: EECs = endometrial epithelial cells; NCS = Nucleolar channel system.

Gerber. Endometrial status at embryo transfer. Fertil Steril Sci 2021. of the “uterine secretome” to find factors predictive of implantative vs. nonimplantative cycles that could be used in clinical practice in the place of available tests that require a mock HRT-FET cycle with endometrial biopsy [25, 31–34]. The present study is a pilot study to establish that NCSs are produced during the typical HRT regimens used to prepare the endometrium for implantation in blastocyst FET cycles by examining uterine secretions.

The study of NCSs in hormonally prepared endometrium dates back 6 decades. In 1963, Clyman [13] used electron microscopy to study the endometrium of normally cycling women vs. those receiving cyclic combined oral contraceptives; however, when we checked their progesterone levels, we noted that the patients with NCSs had elevated progesterone levels, consistent with breakthrough ovulation [16]. In 1979, a study using electron microscopy looked at the endometrium of 7 postmenopausal women aged between 47 and 53 years receiving HRT [33]. These patients were receiving continuous estrogen therapy with cyclic progesterone. NCSs were seen in patients on days 5–7 of progesterone therapy, but not on day 3 or day 10 of progesterone therapy. This was the first study to suggest that NCSs could be produced by HRT without the presence of ovulation. The study did not, however, include any laboratory or sonographic data to definitively rule out spontaneous ovulation, as were included in our study. In 1995, the next and last study looking at the presence of NCSs in the setting of postmenopausal HRT used electron microscopy to correlate the presence of NCSs with the potency of progestins [34]. All women included in the study received conjugated equine estrogen with various cyclic progestins. A biopsy performed on the sixth day of progesterone therapy found that only...
oral norethindrone, norgestrel, and dydrogesterone were able to induce NCSs. On the other hand, vaginal progesterone (100–300 mg) and oral medroxyprogesterone (5–20 mg), which are closer to the formulations used in hormonally prepared FET cycles, were unable to induce NCSs. This study neither included the basic demographic information about the subjects such as age nor any sonographic or laboratory data to confirm the absence of spontaneous ovulation.

NCSs remained largely unstudied for the next 25 years, during which our laboratory discovered molecular markers for NCSs, mostly proteins from the nuclear envelope, enabling light microscopic detection and larger-scale studies (12). Taking advantage of the ease of detection, numerous studies were published, confirming the NCS as a marker of the WOI in both spontaneous and superovulation cycles seen during in vitro fertilization with COH (6, 14–16). With the current rise in HRT-FET cycles, we aimed to re-address whether NCSs were present at the time of FET, which, unlike the prior HRT studies, HRT-FET cycles, we aimed to re-address whether NCSs were present at the time of FET, which, unlike the prior HRT studies, were designed to optimize implantation. In addition, we had ultrasound and serum hormone levels to determine whether NCSs were present before ET on pregnancy outcomes, we intend to study this in a more homogeneous population with good prognoses where clear metrics for pregnancy outcomes can be established. Most importantly, now that we know that NCSs are present and can be identified in endometrial secretions of HRT-FET cycles, the next step is to establish whether the presence or absence of NCSs is associated with pregnancy outcome in these cycles. If true, we will continue to move forward with the ultimate goal of creating a novel point of care test for endometrial receptivity by staining EECs from endometrial secretions for NCSs using the 2-hour method elucidated in this study. This could allow for same-day ET in HRT-FET cycles on the basis of the presence of NCSs as a marker of optimal receptivity.

In conclusion, this is the first report of NCS detection in HRT-FET cycles in the absence of follicular development and ovulation. NCS status can be determined in exfoliated EECs of uterine secretions obtained at the time of ET while maintaining implantation. Our study furthers the goal of establishing whether individualized point of care testing of NCS status in HRT-FET cycles can determine optimal endometrial receptivity and improve pregnancy outcomes.

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