Premature formation of nucleolar channel systems indicates advanced endometrial maturation following controlled ovarian hyperstimulation

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Submitted on May 20, 2013; resubmitted on August 7, 2013; accepted on August 15, 2013

STUDY QUESTION: Is there a shift in the timing of nucleolar channel system (NCS) formation following controlled ovarian hyperstimulation (COH)?

SUMMARY ANSWER: NCSs appear prematurely following COH compared with natural cycles.

WHAT IS KNOWN ALREADY: During natural cycles, NCSs of endometrial epithelial cell (EEC) nuclei are exclusively present during the window of implantation and are uniformly distributed throughout the upper endometrial cavity.

STUDY DESIGN, SIZE, DURATION: Prospective two-cohort study. Cohorts I and II each consisted of seven volunteers for the duration of three menstrual study cycles that were separated by at least one wash-out or rest cycle, between December 2008 and May 2012.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Participants were recruited from a pool of healthy oocyte donors. Consecutive endometrial biopsies were obtained during the same luteal phase on cycle days (CD) 16, 20 and 26 for Cohort I, and on CD14, 22 and 24 for Cohort II, following random assignment to a natural cycle group, a COH cycle group (using a GnRH antagonist), or a COH cycle group receiving luteal phase hormonal supplementation (COH + S). The day of oocyte retrieval was designated CD14 in COH cycles and the day of the LH surge was designated CD13 in natural cycles. Prevalence of NCSs in the nuclei of EECs was quantified using indirect immunofluorescence with an anti-body directed against a subset of related nuclear pore complex proteins that are major constituents of NCSs. Progesterone and estradiol levels were measured on the day of each endometrial biopsy.

MAIN RESULTS AND THE ROLE OF CHANCE: The natural cycle group exhibited peak NCS prevalence on CD20 [53.3%; interquartile range (IQR) 28.5–55.8], which rapidly declined on CD22 (11.8%; IQR 6.3–17.6), CD24 (2.5%; IQR 0.0–9.2) and CD26 (0.3%; IQR 0.0–3.5), and no NCSs on CD14 and 16 defining a short NCS window around CD20. In contrast, in COH and COH + S cycles, NCS prevalence was high already on CD16 (40.4%; IQR 22.6–53.4 and 35.6%; IQR 26.4–44.5, respectively; P = 0.001 compared with CD16 of the natural cycle group, Mann–Whitney), whereas no significant difference in NCS prevalence was detected on any of the other five CDs between the three groups (P > 0.05).

LIMITATIONS, REASONS FOR CAUTION: The cohort size was small (n = 7) but was offset by the all-or-none presence of NCSs on CD16 in natural versus COH and COH + S cycles and the fact that each subject served as her own control.

WIDER IMPLICATIONS OF THE FINDINGS: Premature appearance of NCSs and hence maturation of the endometrium following COH is consistent with previous studies based on histological dating but contradicts studies based on mRNA expression profiling, which reported a lag in endometrial maturation. However, this is the first study of this kind that is based on consecutive endometrial biopsies within the same cycle and that reports such clear-cut differences: no versus robust NCS presence on CD16. Our observation of advanced endometrial maturation following COH may contribute to the reduced implantation rates seen in fresh compared with frozen and donor IVF-embryo transfer cycles. Therefore, the NCS window could serve as a sensitive guide for timing of embryo transfer in frozen and donor cycles.
Introduction

IVF protocols most often involve some form of controlled ovarian hyperstimulation (COH) prior to oocyte retrieval. Consequently, the normal progression of endometrial maturation is disturbed, as determined by histology and gene expression analysis (for review see Bourgain and Devroey, 2003; Horcajadas et al., 2007). Apparently, it is this aberrant maturation of the endometrium that leads to dysynchrony with the developing embryo causing reduced success rates after embryo transfer in the same cycle when compared with embryo transfer in frozen or donor oocyte recipient cycles (Richter et al., 2008; Shapiro et al., 2013). Despite these unwanted consequences, the exact nature of alteration of endometrial maturation by COH is not clear. Whereas histological approaches report advanced endometrial maturation, genomic approaches tend to show a delayed maturation (Kolb et al., 1997; Develioglu et al., 1999; Nikas et al., 1999; Kolibianakis et al., 2002; Mirkin et al., 2004; Saadat et al., 2004; Horcajadas et al., 2005, 2008; Novin et al., 2007; Macklon et al., 2008; Humaidan et al., 2012; Roque et al., 2013). Obviously, it is important to have sensitive markers that inform on the status of the endometrium, specifically the timing of receptivity, i.e. the window of implantation. The gene expression profile identified by the endometrial receptivity array serves as one such marker (Díaz-Gimeno et al., 2011; Garrido-Gómez et al., 2013). Here we evaluate the behavior in COH cycles of a histological marker that sensitively and objectively marks the midluteal phase, the nucleolar channel system (NCS) of endometrial epithelial cells (EECs).

Discovered over half a century ago, NCSs are small (1 μm diameter) organelles in the nuclei of EECs appearing transiently, during an ~5-day window, in the midluteal phase (Dubrauszky and Pohlmann, 1960; Clyman, 1963; Terzakis, 1965; More and McSeveney, 1980; Guffanti et al., 2008). Together with giant mitochondria, subnuclear glycogen deposits and pinopodes, NCSs belong to the ultrastructural hallmarks of secretory transformation of EECs (Morcard and Moricard, 1964; Wynn and Woolley, 1967; Gordon, 1975; More and McSeveney, 1980; Cornillie et al., 1985; Dockery et al., 1988; Spornitz, 1992). By electron microscope, NCSs appear as three layers of membrane tubules embedded in an electron-dense matrix surrounding an amorphous core (Spornitz, 1992). Despite the partial characterization of the molecular composition of NCSs, their function remains as elusive as when originally identified (Kittur et al., 2007; Guffanti et al., 2008). Nevertheless, the identification of protein markers for NCSs now enables their immunodetection at the light microscopic level. Using indirect immunofluorescence, we showed that NCSs are indeed specific to healthy human EECs, that their transient midluteal appearance is robust and independent of fertility status, that they are evenly distributed throughout the endometrial cavity except for the lower uterine segment and that they reside preferentially in the functional luminal layers of the endometrium (Guffanti et al., 2008; Rybak et al., 2011; Szymga et al., 2013). These findings are consistent with a role of NCSs in the endometrial preparation for attachment and implantation of the embryo.

In this study, we exploit NCSs as sensitive indicators for secretory transformation of the endometrium hypothesizing that NCS appearance is shifted in stimulated compared with natural cycles.

Materials and Methods

Subjects

The study was performed in two consecutive legs for each of which seven participants were recruited into two Cohorts, I and II, respectively (Fig. 1A). Participants were from a pool of healthy non-smoking oocyte donors. None had a history of infertility and one in each cohort was parous. Average weight, height and BMI did not differ between subjects of the two cohorts (Table I). Other requirements for inclusion were as follows: (i) age 21–32 years, (ii) CD3 FSH and estradiol (E2) within normal limits (Fig. 2A), (iii) regular 24–35 day cycles, (iv) normal baseline endometrial thickness on ultrasound and (v) willingness to participate in all three groups of the study to allow each subject to act as her own control. The maximum endometrial thickness did not vary significantly between treatments (Fig. 2B). The study was conducted at East Coast Fertility (Plainview, NY, USA) and was approved by the Western Institutional Review Board (Olympia, WA, USA). Informed consent was obtained from all participants.

Cycle characteristics and assignment

Consecutive endometrial biopsies were obtained during the same luteal phase on CD16, 20 and 26 for Cohort I and on CD14, 22 and 24 for Cohort II, following random assignment to a natural cycle group, a COH cycle group or a COH plus luteal hormone supplementation (COH + S) cycle group (Fig. 1). Following participation in the initial cycle, randomization took place between the remaining two groups to determine sequence completion. At least one wash-out cycle separated each study cycle (Fig. 1B). For Cohort I, CD20 was chosen to represent the peak day of NCS abundance in a natural cycle, whereas CD16 and 26 were chosen to fall just beyond the outer limits of known NCS abundance (Guffanti et al., 2008). The endometrial sampling days for Cohort II were selected based on the results from Cohort I. In particular, we probed CD14 to test whether in stimulated cycles NCSs might appear even more prematurely than on CD16 and we included CD22 and 24 to determine whether NCS prevalence in stimulated cycles declined more rapidly, i.e. whether the NCS window expanded or moved in its entirety to earlier luteal days.

The day of oocyte retrieval was designated CD14 in COH cycles and the day of LH surge was designated CD13 in natural cycles. Serial, follicular phase LH measurements and transvaginal ultrasound examinations were used to monitor natural cycles. CD14 was confirmed in natural cycles by documenting follicular collapse on the day after the LH surge. Daily blood and ultrasound monitoring began when the dominant follicle reached about 16 mm in diameter (average of two perpendicular measurements).

IVF protocols

IVF protocols included the possible preliminary use of an oral contraceptive or E2, followed by ovarian hyperstimulation with a gonadotrophin: urofollitropin (Bravette, Ferring Pharmaceuticals, Parsippany, NJ, USA) or recombinant FSH (Gonal-f, EMD Serono, Inc., Rockland, MA, USA; or, Follistim, Schering-Plough Corporation, Kenilworth, NJ, USA); used in conjunction

Key words: nucleolar channel system / ovarian hyperstimulation / endometrial receptivity / window of implantation / immunodetection

STUDY FUNDING/COMPETING INTEREST(S): The study was supported by the March of Dimes Birth Defects foundation (1-FY09–363 to U.T.M.); Ferring Pharmaceuticals, Parsippany, NJ; East Coast Fertility, Plainview, NY and the CMBG Training Program (T32 GM007491 to M.J.S.). We report no competing interests.

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with hMG (Menopur, Ferring Pharmaceuticals). A GnRH antagonist (Ganirelix, Schering-Plough Corporation) was introduced when the lead follicle reached 14 mm in diameter or the serum E2 reached 400 pg/ml. A standard dose of hCG was administered to trigger final oocyte maturation prior to oocyte retrieval. The initial dose of gonadotrophins was determined based on subject BMI and basal antral follicle count. Dose adjustment during the stimulation cycle was based on the ovarian response as evidenced by E2 levels and by size and number of developing follicles. For the former, daily (during the first week of the cycle, every 1–3 days) hormone levels were monitored. The peak levels (Fig. 1C and D) usually occurred on the day of hCG administration but potentially earlier if a patient was at high risk of ovarian hyperstimulation syndrome. For those subjects randomized to receive luteal phase hormonal supplementation, administration of vaginal progesterone inserts (Endometrin, Ferring Pharmaceuticals) 100 mg three times daily was initiated on the day after oocyte retrieval (Doody et al., 2009). Additionally, oral generic E2, 2 mg twice daily, was initiated 6 days following oocyte retrieval (Fatemi et al., 2007).

FSH and LH levels were measured using the IMMULITE 2000 solid-phase, two-site chemiluminescent immunometric assay (Siemens Healthcare, Diagnostics Products Ltd., Llanberis, UK). E2 and progesterone levels were measured using IMMULITE 2000 solid-phase, competitive chemiluminescent enzyme immunoassay.

Endometrial biopsy protocols

Participants received 600 mg of ibuprofen, pre-procedure. All endometrial biopsies were performed using Uterine Explora Model I-MX 120 (Cooper Surgical, Trumbull, CT, USA). Prior to performing each endometrial biopsy, transvaginal sonography was used to map the position of the cervical canal and the endometrial lining. This allowed for easy catheter placement without the need for cervical instrumentation, and for consistent,

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<th>Table I</th>
<th>Characteristics of patients in the study of NCSs and endometrial maturation with COH (mean ± SD).</th>
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<td>Cohort (n = 7)</td>
<td>Weight (kg)</td>
</tr>
<tr>
<td>I</td>
<td>67.1 ± 13.6</td>
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<tr>
<td>II</td>
<td>63.5 ± 11.6</td>
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Figure 1 Participants (A) and study design (B). (A) Each colored dot represents one participant. Cohort I and II participants underwent consecutive endometrial biopsies in the same luteal phase on the indicated CD (black and orange, respectively). Note the color scheme for each patient and CD is maintained in Fig. 3. (B) Study design and treatment assignment for each patient. COH, controlled ovarian hyperstimulation; COH + S, COH plus luteal hormone supplementation.
midline sampling within the endometrial cavity. Endometrial sampling occurred on the anterior surface in anteverted uteri, and on the posterior surface in retroverted uteri to minimize disruption of anatomy. As NCSs are uniformly distributed throughout the upper uterine cavity (Szmyga et al., 2013), the catheter was placed ≥5 cm depth within the uterus, suction was applied and the catheter was retracted from the uterus in a single motion, with the aim of sampling the fundal area and removing as little tissue as possible. The endometrial sample was immediately fixed with 4% paraformaldehyde for 4 h at room temperature and then kept at 4°C until paraffin embedding.

**NCS detection and quantification**

NCSs were detected on paraffin sections by indirect immunofluorescence using a monoclonal antibody (mAb414; Covance Research Products, Princeton, NJ, USA) against a subset of nuclear pore complex proteins that are enriched in NCSs, followed by a fluorescently labeled secondary antibody (DyLight488; Jackson Immunoresearch, West Grove, PA, USA; Fig. 2F and G), as described previously (Guffanti et al., 2008; Szmyga et al., 2013). Briefly, nuclei were identified by double staining with the DNA stain 4',6-diamidino-2-phenylindole (DAPI; Fig. 2E). All samples were imaged on a DeltaVision Core system (Applied Precision, Issaquah, WA, USA) with

![Figure 2](https://academic.oup.com/humrep/article-abstract/28/12/3292/689624)
an Olympus IX71 stand using a 60X/1.42 NA planapo objective and a Cool-Snap HQ2 CCD camera (Photometrics, Tucson, AZ, USA). NCSs in EECs of each biopsy were quantified in 10 fields that were randomly selected regarding NCSs by using the DAPI channel. After selection of the 10 fields, they were imaged automatically and without human intervention across the entire paraffin section in 0.3 μm-step Z-series of optical planes and NCSs were analyzed using our continuous and absolute quantification method (Szmyga et al., 2013). NCSs were identified by visually scanning each optical plane assuring that they were globular rather than tubular structures, i.e. only visible in maximally four to five consecutive planes, allowing for some out of focus light radiating from the 1 μm-diameter organelles (Fig. 2G, arrows). DAPI stained nuclei were counted in maximum projections (Fig. 2E). In this manner, 15 513 NCSs in 64 120 EEC nuclei were counted in 84 endometrial biopsies, assessing between 440 and 1150 (720 ± 149, mean ± SD) EEC nuclei in each. A single observer quantified each sample because the method is observer independent (Szmyga et al., 2013). NCS prevalence was expressed as percentage of NCSs per EECs.

Statistical analysis
All statistical analysis was performed using Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). The distribution of our samples was assessed using D’Agostino and Pearson omnibus and Shapiro–Wilk normality tests. Due to the lack of normal distribution and the small sample size in most cases, the median and interquartile range (IQR) are reported and statistical significance between values was assessed by the Mann–Whitney test. Where mean and SD are reported, statistical significance was tested using an unpaired t-test. Statistical significance was defined as P < 0.05.

Results
NCs appear prematurely following COH
NCSs were identified by their enrichment of a subset of related nuclear pore complex proteins (Fig. 2F and G, arrows and arrowheads) and epithelial cells were counted by their nuclei (Fig. 2E). In the natural cycle group, the window of NCS appearance was very narrow. Specifically, peak NCS prevalence was observed on CD20 (53.3%; IQR 28.5–55.8)—except in 1 patient (Fig. 3A, teal), which was linked to an infection indicated by massive lymphocyte infiltration of the stroma—and rapidly declined on CD22 (11.8%; IQR 6.3–17.6), CD24 (2.5%; IQR 0.0–9.2) and CD26 (0.3%; IQR 0.0–3.5) (Fig. 3A, natural cycle and Table II). No NCSs were detected on CD14 and CD16. In contrast, after COH and COH + S, NCS prevalence was high already on CD16 (40.4%; IQR 22.6–53.4 and 35.6%; IQR 26.4–44.5, respectively; P ≤ 0.001), whereas no significant difference in NCS prevalence was detected between the three groups on any of the other five CDs (Fig. 3A and Table II). Therefore, NCS formation was prominent already on CD16 (Fig. 3A, red arrows). This premature NCS appearance was induced by COH regardless of luteal hormone supplementation.

Increased hormone levels after COH are insufficient to explain NCS induction
Luteal hormone levels for all subjects are depicted in Fig. 3B and C. In natural cycles, median progesterone levels were ≤ 3.0 ng/ml on CD14 and CD16, and increased ≥3.0 ng/ml on CD20 (9.0 ng/ml; IQR 8.5–15.3) and CD22 (9.45 ng/ml; IQR 9.2–12.0) before declining on CD24 (6.3 ng/ml; IQR 4.3–11.0) and CD26 (4.1 ng/ml; IQR 1.2–6.6) (Fig. 3B). In COH cycles, median progesterone levels were
already significantly \((P \leq 0.002)\) increased at CD14 \((8.1 \text{ ng/ml}; \text{ IQR } 6.8–9.5)\) and CD16 \((44.7 \text{ ng/ml}; \text{ IQR } 36.6–75.2)\) remaining supraphysiological on CD20 \((59.3 \text{ ng/ml}; \text{ IQR } 44.1–71.8)\). Thereafter, on CD22 \((3.6 \text{ ng/ml}; \text{ IQR } 2.4–11.1)\), CD24 \((1.2 \text{ ng/ml}; \text{ IQR } 0.6–2.3)\) and CD26 \((0.6 \text{ ng/ml}; \text{ IQR } 0.51.0)\), the levels dropped significantly below those in the natural cycle. In COH + S cycles, the only significant difference in NCS prevalence with that of COH cycles was detected on CD20 \((8.1 \text{ ng/ml}; \text{ IQR } 6.8–9.5)\), where they differed significantly \((P = 0.02)\) (Fig. 3B). No significant differences in peak follicular progesterone levels were noted between the different cycles (Fig. 2C). Significantly, progesterone levels on CD14 in stimulated cycles were comparable to or higher than those observed in natural cycles on CD20 when NCSs peaked, but no NCSs were observed on CD14 \((3.6 \text{ ng/ml}; \text{ IQR } 1.3–7.3; P = 0.02)\) (Fig. 3B). No significant differences in peak follicular progesterone levels were noted between the different cycles (Fig. 2C). Therefore, this NCS surge or spike may only mark a transition in the secretory epithelium, possibly the opening of the time-delimited window of implantation. In stimulated cycles this surge is upshifted and expanded, although the supraphysiological progesterone levels during that period may account for the latter.

NCS presence has been reported to be progesterone responsive (Kohorn et al., 1970, 1972; Roberts et al., 1975; Pryse-Davies et al., 1979). Our data indicate that although increased progesterone may be necessary for NCS formation, e.g. on CD20 in natural cycles, it may not be sufficient because similarly increased progesterone levels fail to induce NCSs on CD14 in COH and COH + S cycles (Fig. 3A and B). While the increase in progesterone (likely acting through transcriptional activation) may precede that of NCS appearance, the lag should be negligible compared with inter-subject variation of timing and given that ultrastructurally indistinguishable organelles, R-rings, form after only hours of transfection of an inducing agent and appear de novo within minutes during live cell imaging (Isaac et al., 1998, 2001; Kittur et al., 2007). Perhaps the supraphysiological E2 levels on CD14 counteract the increased progesterone levels, although they are also present on CD16 when NCSs are prematurely induced in COH and COH + S cycles (Fig. 3A and C).

Our study has several strengths and limitations. Comparing natural and stimulated cycles of the same patients internally controls the study. By assessing NCS prevalence, we home in on the endometrial epithelium, disregarding the stroma. Such a focus may be advantageous to the (mostly opposing) results obtained by morphology (advanced maturation) and gene expression analysis (delayed) (Kolb et al., 1997; Develoglu et al., 1999; Nikas et al., 1999; Kolibianakis et al., 2002; Saadat et al., 2004; Mirkin et al., 2004; Horcajadas et al., 2005, 2008; Novin et al., 2007; Macllon et al., 2008; Humaidan et al., 2012). Using microscopy to quantify NCSs, the results of our study are, perhaps not unexpectedly, in line with those of other histology-based studies that can easily separate epithelium from stroma (Nikas et al., 1999; Kolibianakis et al., 2002; Saadat et al., 2004; Novin et al., 2007). Nevertheless, one might expect a change in gene expression pattern underlying the formation of these remarkable organelles. Possibly, the gene expression profile identified by the endometrial receptivity array is a reflection of this change (Díaz-Gimeno et al., 2011; Garrido-Gómez et al., 2013).

### Discussion

NCSs are robust hallmarks of secretory transformation appearing exclusively during the window of implantation in the nuclei of epithelial cells of healthy human endometrium. Comparing NCS prevalence in sequential endometrial biopsies during the same luteal phase of natural and COH cycles of the same patients, we made two major observations. First, NCS prevalence peaks for a shorter time (CD20 +/− 1 d, i.e. a 3-day window) than determined previously based on biopsies taken during different cycles and from different patients (5-day window) (Guffanti et al., 2008). Secondly, following COH, regardless of luteal hormone supplementation, NCSs peak prematurely already on CD16, when NCSs are not observed in natural cycles. These data support an advanced maturation of the endometrium following COH, which can lead to dysynchrony between the uterine lining and the embryo in fresh embryo transfer cycles, and which may account for increased success rates in frozen and donor oocyte recipient cycles (Richter et al., 2006; Sunkara et al., 2010; Shapiro et al., 2013).

The short window of NCS abundance, narrower than observed previously (More and McSeveney, 1980; Guffanti et al., 2008; Rybak et al., 2011), could be longer due to considerable NCS presence already on CD18, when no biopsies were obtained. However, previous studies report only modest NCS presence on that day (More and McSeveney, 1980; Spornitz, 1992; Guffanti et al., 2008). Although the NCS peak prevalence on CD20 coincides with the beginning of the window of implantation, it fails to cover its entirety, CD20–24 (Wilcox et al., 1999). Therefore, this NCS surge or spike may only mark a transition in the secretory epithelium, possibly the opening of the time-delimited window of implantation. In stimulated cycles this surge is upshifted and expanded, although the supraphysiological progesterone levels during that period may account for the latter.

### Table II

<table>
<thead>
<tr>
<th>CD</th>
<th>14</th>
<th>16</th>
<th>20</th>
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<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>None</td>
<td>0.0 (0.0–0.1)</td>
<td>0.0 (0.0–0.0)</td>
<td>53.3 (28.5–55.8)</td>
</tr>
<tr>
<td>COH</td>
<td>0.0 (0.0–0.2)</td>
<td>40.4 (22.6–53.4)</td>
<td>25.3 (15.8–39.6)</td>
</tr>
<tr>
<td>COH + S</td>
<td>0.0 (0.0–1.8)</td>
<td>35.6 (26.4–44.5)</td>
<td>52.8 (3.5–59.3)</td>
</tr>
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</table>

COH: controlled ovarian hyperstimulation; COH + S: COH plus luteal hormone supplementation; CD: cycle day.
Although the consecutive biopsies in the same luteal phase are a strength of our study, their mere performance and the resulting endometrial injury can influence endometrial histology, clinical pregnancy rates, endometrial gene-expression profiles and levels of midluteal estrogen receptors (Barash et al., 1987; Novotný et al., 1999). Both rely on electron microscopic detection of NCSs and report opposing results. Dehou et al. (1987) find NCSs ‘poorly represented’ in stimulated cycles, whereas Novotný et al. (1999) describe an increase in NCS number and size after hormonal stimulation. In addition to performing non-systematic endometrial sampling, the mean number of epithelial cells with NCSs on CD18–21 was only ~10% in natural and ~20% in stimulated cycles (Novotný et al., 1999). These numbers are below half of the actual number of NCSs observed when counting all NCSs in larger sections of the endometrium, which was made possible by our light microscopic approach (Guffanti et al., 2008; Rybak et al., 2011; Szmyga et al., 2013). Therefore, undersampling could account for the disparate outcomes in those two studies.

Overall, we report on an all-or-nothing change in the endometrial epithelium during the luteal phase caused by follicular COH regardless of luteal hormone supplementation. Therefore, assessment of NCS prevalence provides an objective and sensitive measure for the status of the luteal endometrium, i.e. secretory transformation and initiation of the window of implantation. In support of this statement, we are presently testing the performance of NCS prevalence against that of the gene expression profile identified by the endometrial receptivity array (Díaz-Gimeno et al., 2013).

Acknowledgements

We are grateful to Ed Nejat for analysis of some of the samples and David Kreiner for the support of the study at East Coast Fertility. We thank Nanette Santoro for support at the initiation of the project. All samples were embedded and sectioned at the Histotechnology and Comparative Pathology Facility and imaged at the Analytical Imaging Facility of the Albert Einstein College of Medicine.

Authors’ roles

G.Z. and U.T.M. designed the study, interpreted the results and wrote the manuscript. G.Z. performed all endometrial biopsies. M.J.S. quantified the NCSs in most samples and helped with the statistical analysis. E.A.R. performed initial semi-quantitative analysis of NCSs and contributed to the interpretation of the results. U.T.M. coordinated the study and all authors revised the manuscript and approved the final version.

Funding

Supported by the March of Dimes Birth Defects foundation (1-FY09-363 to U.T.M.), Ferring Pharmaceuticals, Parsippany, NJ, East Coast Fertility, Plainview, NY and the CMBG Training Program (T32 GM007491 to M.J.S.).

Conflict of interest

None declared.

References


Hormone stimulation advances endometrial maturation


Karimzade MA, Oskouian H, Ahmadi S, Oskouian L. Local injury to the endometrium on the day of oocyte retrieval has a negative impact on implantation in assisted reproductive techniques: a randomized controlled trial. Arch Gynecol Obstet 2010; 281:499–503.


More IA, McSeveney D. The three dimensional structure of the nucleolar channel system in the endometrial glandular cell: serial sectioning and high voltage electron microscopic studies. J Anat 1980; 130:673–682.


Sunkara SK, Siozos A, Bolton VN, Khalaf Y, Braude PR, El-Toukhy T. The influence of delayed blastocyst formation on the outcome of...
frozen-thawed blastocyst transfer: a systematic review and meta-analysis. 
Szmyga MJ, Rybak EA, Nejat EJ, Banks EH, Whitney KD, Polotsky AJ, 
Heller DS, Meier UT. Quantification of nucleolar channel systems: 
uniform presence throughout the upper endometrial cavity. Fertil Steril 
Terzakis JA. The nucleolar channel system of human endometrium. J Cell Biol 
1965;27:293–304.

Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus 
Wynn RM, Woolley RS. Ultrastructural cyclic changes in the human 
endometrium. II. Normal postovulatory phase. Fertil Steril 1967;18: 
721–738.
in controlled ovarian hyperstimulation cycles improves implantation rates. 