The nucleolar channel system reliably marks the midluteal endometrium regardless of fertility status: a fresh look at an old organelle

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Objective: To determine whether nucleolar channel systems (NCSs) in the midluteal endometrium are associated with overall fertility status and/or with unexplained infertility.
Design: Retrospective and prospective clinical studies.
Setting: Repository of stored specimens from prior multicenter study and private infertility center.
Patient(s): Retrospective study that included 97 women (49 fertile couples, 48 infertile couples) who had been randomized for endometrial biopsy during the midluteal or late luteal phase. The prospective study included 78 women with a variety of infertility diagnoses.
Intervention(s): Endometrial biopsies were obtained and assessed for the presence of NCSs by indirect immunofluorescence.
Main Outcome Measure(s): The presence of NCS was graded semiquantitatively and dichotomized as normal versus low or absent.
Result(s): Normal presence of NCS was significantly associated with the midluteal phase compared with the late luteal phase (80% vs. 29%). However, there was no association between presence of NCS and fertility status or between presence of NCS and unexplained infertility.
Conclusion(s): Midluteal phase endometrium consistently forms NCSs regardless of fertility status, including unexplained infertility. This indicates a possible role for the NCS in initiating the window of endometrial receptivity. However, the consistent presence of NCSs across several different types of infertility challenges the likelihood that inadequate secretory transformation is a cause of infertility. (Fertil Steril® 2011;95:1385–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: Nucleolar channel system, secretory transformation, receptivity, endometrium, unexplained infertility, immunofluorescence

Fifty years ago, an enigmatic organelle associated with secretory transformation of the endometrium was discovered on the ultrastructural level, and dubbed the nucleolar channel system (NCS) (1). Precise functional and structural characterization of the NCS remains elusive. What is known a half-century later is that the NCS develops transiently in the nuclei of secretory endometrial epithelial cells (EECs) as a membranous organelle of uniform size, ~1 μm in diameter, that is associated with the nuclear envelope and often with a nucleolus. The NCS is comprised of several layers of intertwining membrane tubules embedded in an electron-dense granular matrix that, together, surround an amorphous core (2–5). In a prior work (5), we established a robust method to stain and identify NCSs at a light microscopic level through an immunofluorescence approach using an antibody directed against a subset of nuclear pore complex proteins, a major component of the NCS. Using this method, we determined that NCSs are present in roughly half of all EEC-nuclei during a period preceding and overlapping with the implantation window (i.e., cycle days 19–24 of an idealized 28-day cycle) (5). This 50% prevalence is 10-fold more abundant than previously reported from ultrastructural identification. In addition, we demonstrated that the NCS is specific to healthy, human EECs during the secretory phase. It is not present in proliferative endometrium, endometrial stromal cell nuclei, other hormonally sensitive human tissue such as breast tissue, endometrial carcinoma specimens, or in baboon endometrium (5).

Received June 18, 2010; revised September 3, 2010; accepted October 13, 2010; published online November 10, 2010.
E.A.R. has nothing to disclose. M.J.S. has nothing to disclose. G.Z. has nothing to disclose. M.R. has nothing to disclose. V.E.B. has nothing to disclose. A.J.P. has nothing to disclose. C.C. has nothing to disclose. B.R.C. has nothing to disclose. N.S. has nothing to disclose. U.T.M. has nothing to disclose.
Supported by the March of Dimes Birth Defects Foundation (1-FY09-363 to U.T.M.); CMBG Training Program (T32 GM007491 to M.J.S.); and Ferring Pharmaceuticals, Parsippany, New Jersey (to G.Z.).
Presented at the 57th annual meeting of the Society for Gynecologic Investigation, Orlando, FL, March 25, 2010.
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In addition to its temporal association with the implantation window, the NCS has received significant attention as an important part of normal uterine biology, possibly related to endometrial receptivity. Several observations, derived from multiple ultrastructural studies, support such a role. First, the NCS is induced by P in vivo, whether it is made endogenously or administered exogenously (6–9). Second, the NCS is not found in pregnancy, but remains specific to the midluteal period (2, 10). Third, oral contraceptive (OC) use and intrauterine device (IUD) insertion have been shown to interfere with NCS formation and to prematurely induce its formation during the proliferative phase (11–14). Fourth, administration of high-dose ethinyl E_2 for emergency contraception results in the specific loss of NCSs, whereas glycogen deposits and giant mitochondria, other ultrastructural hallmarks of secretory EECs, develop normally (15). Fifth, controlled ovarian hyperstimulation (COH) increased the number and size of NCSs in the endometrial epithelium of 15 women undergoing IVF compared with those of 15 control women (16). Finally, in several women with unexplained primary infertility lasting from 4.5–8 years, the absence of NCSs was the sole abnormal parameter noted in their secretory endometrium (7, 17). In other cases of unexplained infertility, the development of the NCS was delayed (18).

Endometrial receptivity during the midluteal implantation window in the human menstrual cycle requires secretory transformation of the estrogen (E)-primed proliferative endometrium (19, 20). Characteristic changes heralding secretory transformation result from progressive P exposure, and include the appearance of basal vacuolation—the first histologic evidence of ovulation (21)—and the "secretory triad" of postovulatory ultrastructural findings in the glandular epithelium, namely, glycogen accumulation, the nucleolar channel system, and giant mitochondria (22). Additional changes include pinopode expression on the luminal epithelium (23), the decline of epithelial E and P receptors, although not the stromal P receptors, which are maintained (19), and various genetic and immunohistochemical biomarkers that are specific to a secretory phase endometrium (24–26). Nevertheless, the question remains regarding the extent to which infertility can be attributed to inadequate secretory transformation hindering endometrial receptivity. The multicenter randomized controlled trial by the Reproductive Medicine Network demonstrated that women of infertile couples were no likelier to have an out-of-phase endometrial biopsy—suggestive of inadequate secretory transformation—than were women of fertile couples (27). This finding invalidated the use of classic histologic dating of timed endometrial biopsies for routine fertility investigation and the diagnosis of a luteal phase defect, but as the investigators of the study themselves noted, it did not preclude the possibility that a defect in secretory transformation might cause infertility in at least some instances. And, indeed, the use of Noyes' criteria for classic histologic dating of the secretory endometrium for diagnostic purposes has long been controversial due to the substantial intersubject, intrasubject, and interobserver variability that limit its precision, as well as concerns about the variability introduced by the endometrial sampling procedure (28–31). The availability, however, of a readily detectable, abundant marker of secretory transformation, the NCS (5), enables a fresh look at the relationship between inadequate secretory transformation and infertility.

Based on the ultrastructural data showing the NCS to be directly relevant to endometrial receptivity (6–18, 32), we hypothesized that the presence of NCS would vary by fertility status and by specific infertility diagnosis. Therefore, our objectives were as follows: first, to confirm the association of the NCS with the midluteal phase; second, to determine whether the presence of NCS is associated with overall fertility status; and third, to determine whether the presence of NCS is specifically associated with unexplained infertility.

MATERIALS AND METHODS

Participants

Endometrial biopsies were obtained from two sources. The first source is the repository of the National Institute of Child Health and Human Development–sponsored Reproductive Medicine Network (RMN) at 2 of the 12 academic centers that participated in the original study (27) and that had a research consent form allowing for future research on the specimens, site A (University of Pennsylvania) and site B (University of Texas-Southwestern Medical Center). After the study was approved by the respective institutional review boards at the Albert Einstein College of Medicine and the two RMN sites, 107 endometrial biopsies were received, 97 of which contained sufficient glands for NCS scoring. Among the site A specimens, stratification by luteal phase timing and fertility status, revealed no statistically significant differences in age, racial composition, fertility status, or biopsy timing (see Supplemental Material). Second, 78 endometrial biopsies were obtained, during a natural cycle and without hormonal medication, from patients with various infertility diagnoses from site C (East Coast Fertility, a private fertility center in Long Island, NY), with institutional review board approval. Endometrial biopsies from sites A and B were processed as previously described (27) and preserved as frozen or paraffin sections. Site C specimens were obtained using a Pipelle suction catheter, formalin fixed, and paraffin embedded, as we described previously (5). For background and cycle information see Supplemental Material.

NCS Imaging and Scoring

Immunostaining was performed essentially as described (5) (see Supplemental Material). Epifluorescent detection and scoring of NCS prevalence was performed on an Axioskop II light microscope (Zeiss, Oberkochen, Germany) using a 63×/1.4 NA planapo objective. The prevalence of NCSs was graded semiquantitatively as normal, low, and absent (Fig. 1) according to criteria established previously with a training set of biopsies, in which the absolute number of NCSs was determined (5). As observed previously, NCSs appeared and disappeared rapidly within 1 day (i.e., they were either abundant or they were low or absent) (5, 32, 33). Therefore, the data of the low and absent categories were combined for binarization. Designation as normal required the presence of NCSs in >10% of epithelial cell nuclei in at least two distinct regions of the specimen. We previously determined the 10% cutoff using absolute numbers of NCSs (5). The purpose of this study was to establish NCS presence versus absence. To quantify NCSs, stereology could be applied (34, 35), although that would be challenging for such a large sample set. Nevertheless, only a few samples approached the 10% cutoff, and most were far above or below. Specimens with fewer NCSs in an entire section with an average of 50–100 glands were graded as low. All sample preparation, immunodetection, and scoring was performed by at least two observers who were blinded to the clinical information associated with each biopsy specimen. Among the specimens (n = 175) analyzed, 10.9% received discrepant scores and were reevaluated by a third referee, also blinded, for final grading. This interobserver difference can be explained by slight variations in procedure and identification of NCSs.

Supplemental Material Online

For additional Material and Methods, including outcome measures and statistical analyses, see Supplemental Material online.

RESULTS

Consistent with our prior results (5), the presence of NCS was far greater in the midluteal (80%, n = 30) compared with the late luteal phase (29%, n = 31; $P<.001$; Table 1). When these groups were stratified by fertility status, the association persisted (Table 1). Endometrial specimens from fertile compared with infertile couples demonstrated similar NCS presence (55.1% vs. 52.1%, respectively;