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Comparing cervical mucus changes in response to an oral progestin or estrogen withdrawal in ovarian suppressed women: a clinical pilot

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Abstract

Purpose—Prior studies evaluating the effect of administered progestogens on peak cervical mucus have not controlled for the influence of endogenous hormones. To address this, we treated women with a gonadotropin-releasing hormone (GnRH) agonist to suppress the hypothalamus-pituitary-ovarian (HPO) axis and used transdermal estradiol replacement to stimulate peak cervical mucus, and then evaluated the effects of an oral progestin or estradiol withdrawal.

Materials and Methods—We used a crossover design to examine cervical mucus changes in women receiving transdermal estradiol replacement following intramuscular administration of leuprolide acetate. After increasing estradiol patches to mid-cycle levels, subjects were assigned to either 0.35 mg oral norethindrone with continuation of the patches (NET) or estradiol withdrawal by patch removal (E2WD). We collected serum and cervical mucus samples at 0, 2, 4, 6, 22 and 24 hours following the intervention.

Results—We analyzed 12 cycles (6 NET, 6 E2WD) from three subjects. Baseline cervical mucus scores were favorable to sperm penetration [NET median 11, interquartile range (9–12), E2WD 13 (12–13)]. Two hours after removal of estradiol patch or administration of norethindrone, cervical mucus scores declined [NET 8.5 (4–9), E2WD 10.5 (10–12)]. Low cervical mucus scores persisted at 24 hours with NET [8.0 (7–8)] but not E2WD [10.5 (8–11)].

Conclusions—We observed a rapid decline in cervical mucus Insler scores following administration of a single dose of oral norethindrone, and scores remained lower and unfavorable through 24 hours. Estradiol withdrawal did not result in similar unfavorable changes.

Keywords

Cervical Mucus; Contraception; Insler Score; Norethindrone; Mini-Pill; progestin

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Disclosures of Interest

The authors report no conflict of interest.

1. Introduction

The majority of reversible contraceptive users in the United States and approximately a quarter of women worldwide use a progestin-containing method of contraception [1,2]. The contraceptive effects of progestins include dose-dependent effects on the hypothalamus-pituitary-ovarian (HPO) axis, endometrium, tubal motility and cervical mucus [3,4]. Low-dose progestin-only methods such as the progestin-only pill (POP) and levonorgestrel intrauterine system (IUS) work by thickening cervical mucus [5].

Cervical mucus fluctuates throughout the menstrual cycle, with sperm permissive characteristics peaking around ovulation in response to increasing estradiol (E2) levels, followed by reversal of those characteristics after ovulation when progesterone (P4) replaces E2 as the dominant sex-steroid [6]. The current clinical standard for appraising cervical mucus is the cervical mucus score (ie. Insler score) that examines mucus based on 5 metrics including volume, spinnbarkeit (stretch), ferning, viscosity and cellularity on a 15 point scale (see Table 1) [7]. Per WHO guidelines, scores above 10 are considered “mucus favoring penetration” and scores below 10 are considered to be “unfavorable to penetration.”

Contraceptive drug studies that aim to evaluate the pharmacodynamics changes in cervical mucus must evaluate the effect at the time of peak ovulatory mucus otherwise the sampling may reflect the combined effect of the drug treatment and either inadequate E2 levels or endogenous P4 effects. To clarify the effects of estrogens and progestogens on cervical mucus, we developed an artificial cervical mucus cycle model and used this to evaluate mucus changes in response to hormonal manipulation [8]. Our objective was to compare the qualitative and temporal changes of cervical mucus following estradiol withdrawal versus the administration of 0.35 mg oral norethindrone. Based on prior literature, we hypothesized that administration of norethindrone in women with favorable mid-cycle mucus would result in a rapid (by 2 hours) change to a thick unfavorable mucus (cervical mucus scores <10).

2. Methods

2.1 Subjects

We conducted a crossover study in healthy women between 21 and 40 years old with a history of normal menstrual cycles (between 21–35 days without metrorrhagia). Relevant exclusion criteria included: history of anovulation, use of injectable contraception (depomedroxyprogesterone acetate) within 12 months or any other hormonal contraceptive or hormonal supplement within the prior 2 months, use of any hepatic inducing drugs, current cervical infection or evidence of abnormal cervical cytology. We excluded users of copper IUDs as these may increase cellularity and protein content related to inflammatory effects from the copper ions [9]. Subjects were told to report any new drug use. This study was reviewed and approved by the Oregon Health & Science University institutional review board and registered on [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02969590) (NCT02969590).

2.2 Hormone Replacement

Figure 1 summarizes our study design. In order to qualify for the intervention phase of our study, subjects needed to demonstrate favorable mucus at ovulation (Insler score of ≥ 10

within 24 hours of an LH surge) and progesterone level in the luteal phase consistent with ovulation (a single P4 of > 3 ng/ml between days 18–35 [10]). Following this spontaneous cycle, participants received a single intramuscular injection of leuprolide acetate (11.25 mg, Lupron®; Abbvie, Chicago, IL), a dose documented to suppress ovarian function for at least 3 months[11]. After confirmation of ovarian suppression (serum E2 < 35 pg/ml 21–28 days post-injection), subjects initiated estrogen replacement with transdermal (TD) E2 patches (0.1 mg/day Vivelle-Dot®, Noven Pharmaceuticals Inc, Miami, FL), changed twice weekly. This dose restores circulating E2 levels in ovarian-suppressed women to 89 ± 38 pg/ml[12], levels comparable to the late follicular and luteal phases of natural cycles[13,14]. Patches achieve peak concentrations by 12 hours, have a half-life of 5.9–7.7 hours and return to baseline levels with 24 hours of removal[15].

At the completion of the study, subjects received 5 days of oral micronized progesterone (Prometrium 200 mg, Abbvie) [16] to induce a withdrawal bleed, and then received a low dose oral contraceptive pill (0.35 mcg ethinyl estradiol/1 mg norethindrone acetate, Allergan, Irvine, CA) for at least one cycle to ensure hormone replacement and contraception during resolution of the hypogonadism from the leuprolide acetate.

2.3 Timed mucus collection

We admitted subjects to the OHSU clinical research observation unit for timed evaluations of cervical mucus during four separate evaluations. On the day prior to each admission, we instructed subjects to apply 3 fresh E2 patches (0.3 mg/day) to achieve mid-cycle level E2 effects[14]. Levels of E2 in normal women during the mid-cycle surge have been reported to be 335 ± 21 pg/ml [14] or approximately 3 times the E2 concentrations measured with a 0.1 mg/day transdermal E2 dose [12]. On the day of the first and third admission, the study coordinator randomized each subject to the experimental condition, either: 1) oral progestin administration (e.g. a single dose of 0.35 mg Norethindrone) (Ortho Micronor, Janssen Pharmaceuticals, Titusville, NJ) [17] with continuation of the E2 patches (Treatment arm=NET); or 2) estradiol withdrawal (Treatment arm= E2WD) (e.g. removal of the patches). The subject then received the converse intervention for admissions two and four so that each subject would undergo each condition (NET vs E2WD) twice. The clinician responsible for collection and assessment of the mucus samples remained blinded to the intervention condition. Following each admission, subjects resumed treatment with the 0.1 mg E2 patch until their next scheduled inpatient assessment. We required a minimum of five days at this level before repeating the dose escalation and the next evaluation.

During each admission, we collected both serum and mucus samples at 0, 2, 4, 6 hours following the intervention and then again at 22 and 24 hours the following day. The intervention condition (e.g. patch removal or norethindrone administration) occurred immediately following the 0-hour sample. To collect mucus, we used a vaginal speculum to expose the cervix and a synthetic scopette (Puritan Medical Products) to gently cleanse the external os. Following this, we inserted a Unimar Aspirette [18](Copper Surgical) device approximately one centimeter into the os before applying suction to obtain a sample. We immediately evaluated the mucus sample for Insler score. All of the investigators who

evaluated specimens had prior experience scoring cervical mucus and received standardized training in mucus appraisal for this protocol.

We collected whole blood from an intravenous catheter, used a refrigerated centrifuge to separate serum, and then stored samples between -70 to -80°C until analysis. The Endocrine Technologies Support Core (ETSC) at the Oregon National Primate Research Center (ONPRC) used a Roche cobas e411 automated clinical platform (Roche Diagnostics, Indianapolis, IN) to measure E2, P4 and norethindrone. The assay ranges for the E2, P4 and norethindrone assays were $5 - 4300$ pg/ml, $0.030 - 60$ ng/ml, $0.039 - 10$ ng/ml respectively. Intra- and inter-assay CVs for the Roche assays in the ETSC are consistently less than 9% [19,20].

2.4 Data Analysis

We designed this study as a small clinical pilot given the highly experimental design and lack of previous studies guiding estimated effects. We chose to recruit five participants to account for biological variability and dropout. Our primary goal was to compare differences in cervical mucus score between the estradiol withdrawal and norethindrone treatments at each time point. Secondly, we sought to compare E2, P4 and norethindrone levels for each treatment arm. For both primary and secondary comparisons, we used descriptive statistics with 95% confidence intervals. We did not perform formal hypothesis testing, due the small sample size and lack of independence in repeated observations. Descriptive analysis was conducted using R version 3.5.0 (R Core Team (2018), Vienna, Austria).

3. Results

We enrolled five healthy women with normal cervical mucus scores and hormone profiles during natural ovulatory menstrual cycles. One subject did not demonstrate ovarian suppression (e.g. $\text{E2} < 35$ pg/mL) five weeks following leuprolide, and therefore did not continue through the intervention period (see Figure 2). The remaining four participants demonstrated ovarian suppression following leuprolide acetate [median E2 at 3 weeks 15.8 pg/ml; range (6.0–31.4)] and completed all four inpatient visits. After reviewing our data, we excluded all observations from subject 4, who had baseline (time=0 hours) E2 levels below 40 pg/mL, suggesting noncompliance with the patch schedule. The demographic characteristics of the three analyzed subjects are shown in table 2. Complete E2, P4, NET levels and mucus scores for all three analyzed subjects are reported in supplementary table 1.

3.1 Hormone evaluations

For all subjects, treatment with the 0.3 mg patch dose resulted in midcycle concentrations of E2 at the 0-hour (baseline) sample [E2WD admission median 563 pg/ml, range (198–684); NET admission 223 pg/ml, range (135–378);][13] (See Figure 3). Progesterone concentrations remained at follicular phase levels in all subjects at all time points. As expected, E2 levels dropped rapidly following patch removal [median 45 pg/ml, range (34–90) by 6 hours] but remained elevated following administration of norethindrone [265 pg/ml, range (68–414)] (Fig 3). Norethindrone levels were undetectable at the start of all

interventions (0-hours). Treatment with norethindrone resulted in peak norethindrone levels at the 2-hour sample interval, and these declined rapidly in all subjects to the non-detectable range by 24 hours (Figure 4).

3.2 Cervical Mucus

Cervical Mucus scores declined from baseline following patch removal or administration of oral progestin in all of the interventions (see table 3). Baseline cervical mucus scores were favorable in both groups [median 13 [interquartile range (IQR) (12–13)] (E2WD) and 11 [IQR (9–12)] (NET)]. At 2 hours, cervical mucus scores following norethindrone administration were <10 (median 8.5 [IQR (4–9)], but not for E2WD (10.5 [IQR (10–12)]). For both treatment conditions, median cervical mucus scores reached a nadir at the 6-hour observation [median 8.5 [IQR (8–9)] (E2WD) and 6.5 [IQR (6–8)] (NET)]. At 24 hours, cervical mucus scores increased in both groups but remained <10 for the NET group (median 8 [IQR (7–8)]) and were >10 in the E2WD group (10.5 [IQR (8–11)]). When we examined the viscosity (consistency) of mucus specifically, scores were similar at baseline (median 3 [IQR (3–3)] in E2WD and 3 [IQR (2–3)] in NET) and remained similar throughout the experiment.

Discussion

We present the first report of evaluation of mucus changes in response to oral norethindrone contraception or estrogen withdrawal following leuprolide injection with concurrent estradiol patch administration. Prior studies of cervical mucus changes following administration of a contraceptive progestin have not accounted for the dynamics of natural hormonal changes in natural cycles. Using a GNRH agonist and estradiol patches, we experimentally created ovulatory E2 levels and mucus consistent with midcycle at the start of our evaluation. We could then evaluate the effect of oral administration of a 0.35 mg norethindrone pill over 24 hours while estradiol levels remained high, a time of peak pregnancy risk during natural cycles.

Most previous studies of progestin-only contraception have not adequately controlled for cycle effects [21]. In the case of the POPs, clinical guidelines recommend a daily dosing as close to 24 hours from the previous dose as possible. This derives from limited clinical data describing diminishing cervical mucus effects by this time. The only evidence that directly supports this guideline is a study from Cox et al., which includes a single figure displaying mucus results from six normal cycling women taking 0.5 mg of megestrol [22]. They found that sperm penetration of cervical mucus appears to decline two hours after ingestion and return to normal around 22–24 hours. However, these samples were collected in the peri-ovulatory interval, thus mucus changes were potential confounded by ovulation.

Compared to the Cox et al. study, our design has many strengths. We evaluated a similar number of subjects at the same time points with additional information about serum drug levels. In our study, norethindrone levels peaked at 2 hours following ingestion, and, similar to Cox et al, we saw substantial mucus changes by this time point. Our peak norethindrone levels are consistent with previous PK analysis of the same 0.35 mg pill by Atrio et al. [23] and Prasad et al. [24]. Unique to our study, we have additional serum levels at 22 hours that

show norethindrone values already near zero. However, unlike the Cox study, we did not see a rapid return to baseline scores by 24 hours [median 11 IQR (9–12) (0 hours), 8 IQR (7–8) (24 hours)]. Thus, our data supports the current theory of a rapid change leading to unfavorable cervical mucus in response to oral administration of norethindrone. However, our results do not support the need of a strict 24-hour dosing regimen for the mini-pill and suggest mucus production continues to be altered even after drug levels are undetectable[25]. Future studies should consider assessing cervical mucus at further time points to see how long beyond 24 hours these changes persist.

We recognize several limitations to this study. Biological variability in steroid absorption or metabolism may have affected results in our small clinical experiment. We were unable to completely control for substances such as alcohol, antihistamines and ascorbic acid that could alter sex-steroid metabolism [26]. However, we used a crossover design that allowed interventions to be tested in the same individual. Second, due to study burden and logistical considerations, we were limited in the frequency, type, and timing of repeated mucus measurements. In order to limit visits, we did not include a third arm with an estradiol maintenance control. Study burden may have contributed to the non-compliance of patch use in subject 4. The number of evaluations also influenced our need for several examiners to cover the research unit, leading to the possibility of inter-observer differences. However, during the standardized training prior to the study, all four study clinicians conducted blinded appraisals of mucus samples and established consensus interpretation of criteria. Future contraceptive studies may want to consider evaluation of mucus using either a shorter acting gnRH agonist or use of an oral antagonist to limit study interval [27]. Finally, we did not conduct sperm penetration testing as the laboratory procedures necessary to assess fresh samples were impossible given the number of samples we had each admission. Sperm penetration testing has not been validated as measure for contraceptive effect[21].

Our small clinical pilot was not designed with sufficient power to make the application of formal statistical hypothesis tests meaningful. However, we observed rapid changes in norethindrone administration and relatively smaller and slower changes in the E2WD group over 24 hours. This suggests the possibility that the mechanism of steroid regulation of cervical mucus changes are not the same between estrogens and progestogens. Clarifying these mechanisms may help us understand contraceptive mechanism of progestins at the level of the cervix during non-conventional clinical scenarios such as extended usage of contraceptive implants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:

Study Design: Subjects were followed during a spontaneous cycle to verify ovulation (luteal phase progesterone ≥ 3 ng/ml) and production of favorable peri-ovulatory mucus (Insler score ≥ 10). Participants then underwent an intramuscular injection of leuprolide acetate and confirmation of ovarian suppression at 21–28 days post-injection. Subjects then received estrogen replacement with transdermal estradiol (E2) patches (0.1 mg/day). Estradiol replacement was increased to 0.3 mg/day one day prior to interventions (estradiol withdrawal or 0.35 mg oral norethindrone with estradiol maintenance) and sampled for both serum and mucus at 0, 2, 4, 6, 22, and 24 hours following intervention. Each participant underwent each intervention arm twice with at least five days of washout time between interventions. E2: Estradiol

Figure 2:

Study flow diagram and intervention order for all four completed subjects. * Subject 4 was not included in the analysis because estradiol (E2) levels were consistent with patch non-compliance.

Figure 3:

Serum estradiol (E2) levels for 3 participants from 0 to 24 hours for 4 cycles each (total 12 cycles). In the estradiol withdrawal group, estradiol patches (0.3 mg/day) were removed at time=0. In the norethindrone group, patches were worn, but participants were given 0.35 mg of oral norethindrone at time=0.

Figure 4:

Serum norethindrone (NET) levels for 3 participants from 0 to 24 hours following administration for 2 cycles each (total 6 cycles). Participants were given 0.35 mg of oral norethindrone at time=0.

Figure 5:

Total mucus score over time by participant and study condition. Scores are calculated as within-participant averages from 2 observations per participant for each study condition.

Table 1.

Modified Insler Score from the WHO laboratory Manual for the Examination and Processing of Human Semen [7].

Score:	0	1	2	3
Volume	0 ml	.01–.1 ml	.1–.29 ml	= > .3 ml
Viscosity	thick, highly viscous,	intermediate viscosity	mildly viscous	watery, minimally viscous
Ferning	no crystallization	atypical fern formation	primary and secondary stem	tertiary and quaternary
Spinnbarkeit	<1cm	1–4 cm	5–8 cm	9 cm or more
Cellularity	>20 cells/HPF	11–20 cells/HPF	1–10 cells/HPF	0 cells

Table 2:

Demographic data for all three subjects

Subject	Age	Gravida	Parity	BMI	Race/Ethnicity	Smoker	Medications	Other Health History
1	40	0	0	27	White	No	Amino acid mood supplement	
2	33	1	0	19	White	No		Tubal Ligation
3	29	2	2	23	White	No	Acyclovir as needed for oral herpes	Von Willebrand disease without menorrhagia

Table 3:

Median cervical mucus scores for all three subjects by intervention (estradiol withdrawal vs. norethindrone administration with estradiol continuation) at 0 (Baseline), 2, 4, 6 and 24 hours. Six measurements (2 from each woman) contribute to each group median for every time point.

Time Point	Estradiol Withdrawal Median (interquartile range)	Norethindrone Median (interquartile range)
Baseline	13 (12, 13)	11 (9, 12)
2 hours	10.5 (10, 12)	8.5 (4, 9)
4 hours	10 (9, 10)	8.5 (7, 9)
6 hours	8.5 (8, 9)	6.5 (6, 8)
22 hours	10.5 (9, 12)	8.5 (6, 10)
24 hours	10.5 (8, 11)	8 (7, 8)