OFFICIAL TITLE:
Regulation of Cervical Mucus Secretion

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**Project Summary**

Hormonal contraception remains the primary form of contraception used by US women. These methods include oral contraceptive pills, rings, patches, injectables, implants and the progestin containing intrauterine device (IUD). All of these progestin-based hormonal methods are thought to cause thickening of the cervical mucus that causes poor sperm penetration. While it is widely accepted that progesterone is a mediator of mucus changes observed at midcycle, the regulation of this effect is not well understood. Subsequently, lingering questions regarding the onset and extent of progestins actions on cervical mucus affects our prescribing practice of current progestin-based contraception. Moreover, our neglect of cervical mucus over the last 40 years has perhaps led us to overlook a biologically intuitive target for non-hormonal contraceptive development.

Most of our current knowledge of cervical mucus remains based in clinical studies done in the 60s and 70s with the advent of various forms of progestin only contraception such as the mini-pill. However, these studies suffer from serious limitations. First, mucus changes progressively under the influence of increasing amounts of estrogen by becoming less viscous and more favorable to sperm penetration. Mucus evaluation of a progestin must be timed to coincide with the peak effect of endogenous estradiol and potential trough effect of the contraceptive steroid, a complex problem for many study designs. Additionally, ovulation presents an important confounder that is not accounted for in many of the mini-pill studies when ovulation occurred 15-70% of the time. With ovulation there is also endogenous progesterone being produced, subsequently suppressing endogenous estradiol production. As there is a convolution of forces on mucus from both estradiol withdrawal and endogenous progesterone, the early and late effects of progesterone signaling remain incompletely understood.

We propose studies to evaluate the temporal changes in human cervical mucus in response to progesterone and progestins as an initial step toward clarifying these relationships. Our hypothesis is that direct effects of progesterone on the endocervix, independent of estrogen withdrawal, cause contraceptive changes to cervical mucus. To study this, we plan to conduct a randomized, prospective, crossover study examining cervical mucus changes in a small cohort of women in whom we will suppress circulating hormonal levels by administering a GnRH agonist. We will then artificially replace Estrogen and Progesterone in order to differentiate their effects on clinical and laboratory measurements of mucus quality. We will be looking closely at the immediate changes in mucus when .35 mg of norethindrone, a marketed drug is administered in this experimental setting. We will also collect cervical cell samples at various time points and perform RT-PCR to determine whether genes for membrane bound progesterone receptor are expressed and regulated by Estrogen and Progesterone.

We believe this research will offer important, immediate insight into progestin regulation of cervical mucus. However, we also expect this to lay important foundations for future studies both clinical and laboratory based. We hope our model will provide the vigorous conditions with which we can evaluate mucus effects from other progestin-based contraceptives such as the levonorgestrol-releasing intrauterine system or the etonorgestrel implant. Last, elucidation of the relationship between membrane bound progesterone receptors and cervical mucus will offer new biochemical pathways to target for contraceptive development.
1. DESCRIPTION OF THE PROJECT

1.1 Rationale and objectives of the study

1.1.1 Rationale

At this time, the majority of women in the United States using contraception rely on short acting, reversible, hormonally-based contraceptive methods. More than a quarter rely on combined oral contraceptive pills; this increases to 35% when other hormonal methods that inhibit ovulation (injectables, rings, patches and implants) are included[1]. Although there are many peripheral benefits to the use of hormonal contraception, for many women the perceived risks of hormonally based contraception outweigh perceived benefits[2][3][4]. A smaller, but no less important group of women have relative (e.g. obesity) or absolute contraindications to hormonal contraception due to thrombosis risk associated with estrogen [5]. It is widely believed that estrogen-free hormonal methods that do not block ovulation (e.g. norethindrone progestin-only pill; LNG-IUS) work at least in part by inhibiting cervical mucus [6–9]. Although these methods exploit the progesterone-mediated changes in cervical mucus, we know little about how progesterone regulates this effect. We have the potential for developing an easy to use, non-hormonal contraceptive based on mucus thickening alone with a better understanding of the regulation and modification of this secretory machinery.

Cervical mucus is thought to rely on a careful balance of its solid (primarily mucin proteins) and liquid components to give it the qualities that make it either facilitate sperm transport into the uterus or help it serve as a barrier to sperm and other pathogens. Mucus production occurs in the endocervix where a single layer of columnar cells line the endocervical canal, producing a mixture of water, ions, lipids, mucin proteins and non-mucin proteins. Mucin proteins are large glycoproteins, with highly anionic carbohydrate ends that bind liquid and form polymer networks which either trap microorganisms (including sperm) or facilitate sperm transport depending on their orientation [10–12]. Despite forming the critical backbone to the gel-like quality of mucus, mucins comprise only 2-5% of the mucus content by weight while water content ranges from 90% when mucus is pre- or post-ovulatory, and up to 98% at midcycle[13][11]. It appears that a number of ion channels are involved in regulation of fluid content in mucus secretion including sodium channels, calcium-based chloride channels, aquaporins and the cystic fibrosis transmembrane conductance regulator (CFTR)[10,14].

The production of mucin protein genes and the flow of water and channel ions has been shown to be directly correlated to estrogen and progesterone levels[12,14,15]. However, to date, no direct mechanistic pathway exists describing the steroid regulation of mucus production. We know estrogen promotes mucin gene transcription and protein levels as well as fluid transudation and ion exchange[15,16]. Meanwhile, these effects are all muted with the presence of progesterone in the luteal cycle. Experiments using both anti-estrogens and synthetic progestins demonstrate that these compounds affect mucus quality and sperm penetrability [17–20]. However, it remains unclear what changes to cervical mucus result from inhibition or withdrawal of estrogen-based effects and what changes derive from direct progesterone actions on endocervical cells. Previous experiments of progesterone-based contraceptives were done in normal cycling women where drug treatment occurs in the setting of endogenous hormone production.

Steroid regulation of mucus likely affects multiple components of the cellular machinery at various time points in the synthesis and secretion of cervical mucus. Some experiments monitoring mucus changes following ovulation (or exogenous administration of progesterin) demonstrate changes in quantity and quality much faster than what would be expected from the
classical mechanisms through which steroids are thought to work. In the well-characterized, classic nucleus-based paradigm, progesterone and estrogen bind to receptors located in the cytosol, but must be transported into the nucleus where they bind to the hormone response element (HRE) located within specific gene promoter regions and activate transcription [21]. This is generally a “slow” process (e.g. several hours to days) as it relies on DNA transcription and RNA translation to produce proteins that promote cellular changes. However, there is evidence suggesting changes in cervical mucus happen very rapidly. Serum contraceptive steroid levels peak approximately 2 hours after oral ingestion [22]. One study demonstrates cervical mucus reaching its maximum unfavorability 3-4 hours after ingestion, and others studies show the arrest of sperm migration to occur within 30 minutes to 2 hours [7,23]. While these older studies are limited in that progestin administration does not coincide with peak estrogen effects on mucus, the demonstration that cervical mucus changes occur faster than other progesterone mediated effects on the reproductive system suggests that possibly direct (non-nuclear) pathways are involved [7]. At least two classes of membrane associated progesterone receptors (MAPRs) have been described; the progesterone membrane component 1 and 2 (PGRMC1, PGRMC2) and the membrane progestin receptors α and β (mPRs) [21]. These receptors have been shown to bind progesterone and carry out functions in other reproductive tract tissues through non-nuclear mechanisms. However, whether progesterone could be involved with the inhibition of an ion channel or block the exocytosis of secretory granules of the cervix is completely unknown[15,24].

Clarifying progesterone’s regulation of mucus would have large implications on our evaluation of cervical mucus as a contraceptive target. While many current methods rely on mucus thickening for contraception, the potency, timing, and mediation of these transformations are poorly understood. Answering these questions will contribute to the improvement of existing hormonal approaches and provide insight into new targets for non-hormonal contraceptive methods.

1.1.2 Objectives and hypothesis

**Hypothesis:** Contraceptive action of progestogens occur through direct effects on cervical mucus and not indirectly though reduction in systemic estrogen.

**Primary Objective:** Using ovarian suppressed women, we seek to individually evaluate the effect of E_2_ and progestin on cervical mucus to better understand its hormonal regulation. To do this we will:

1. Create and validate an artificial model of the menstrual cycle using exogenous E_2_ and P_4_ replacement in ovarian suppressed women.
2. Evaluate effects of increasing estrogen levels on cervical mucus.
3. Evaluate the timing of both the onset, duration and termination of progestin actions on cervical mucus following estrogen priming.
4. Compare the effects of estrogen withdrawal to the synthetic progestin norethindrone.

**Secondary Objectives:**

1. To determine if membrane bound progesterone receptors are present in the cervix and are regulated by E_2_/ P_4_.
1.2 Previous Similar Studies

Much of the literature examining effects of progestins on cervical mucus originate from the 1960’s and 1970’s during the advent and introduction of progestin-only contraception. These studies all had similar methods [18,23,25–31]. After baseline measurements were taken, a group of normal cycling women were started on a progestin-only treatment one of the first few days of the following cycle. Laboratory and clinical measures were assessed serially with varying degrees of frequency including serum and urine hormonal levels to assess hormone profiles, basal body temperature, vaginal cytology, endometrial morphology and mucus characterization through qualitative changes (Insler scoring) and sperm-mucus interaction (through either in vitro penetration testing or post-coital testing). Findings consistently demonstrated changes in mucus characteristics with ovulation including lower spinnbarkeit scores, poor ferning, increases in viscosity, greater cell content and decreases in volume [18,25,28]. Results with respect to sperm penetration were mixed, as some tests used a post-coital test while others used an in vitro sperm penetration. While some studies demonstrated essentially zero penetration at peak progestin levels following administration [23,26,31], others demonstrated decreased penetration, but with some failures [25,28,31,32].

In addition to mucus changes, these studies demonstrate that progestin-only pills had a range of effects including suppression of proliferative activity in the endometrium, suppression of midcycle FSH and LH peaks, and suppression of ovulation in 15-70% of cycles[7,18,20,32]. An important feature of our proposed study involves eliminating the variability due to the timing of natural ovulation through the use of artificial cycles. The progestin intervention occurred at variable times when mucus may not have been at its most favorable. Moreover, since ovulation is not fully inhibited, there are likely endogenous progesterone effects in addition to exogenous progestin effects. No previous studies disentangle this confounding influence on cervical mucus.

The other advantage of our methodology is it allows reappraisal of the time course of progestin effects. Currently, the commonly held belief among providers is that progestin effects have rapid onset and rapid offset. In reality very few studies examine this outcome and even then, in those older studies, rigid study design is not employed. The study by Cox et al. evaluating six users of .5 mg of oral megestrol acetate showed that mucus penetration sharply falls from full penetration to no penetration in 2 hours, followed by an equally steep return to penetrative mucus at 22-24 hours[23]. Cox’s study contains almost no measures of statistical significance only pooled data of the 6 subjects is presented. Lebech et al., examining 17 patients, administered .25mg of megestrol acetate orally and noted “normal penetration of sperm” 38 hours later. In addition to no statistics, or measurements of sample variation, Lebech’s study also noted anovulatory cycles in some of his controls.

Perhaps more interesting than the literature reported is the absence of literature supporting current guidelines of oral administration. The most definitive review on progestin only oral contraceptives is a 195 page supplementary report published in Contraception in 1994 by McCann and Potter[22]. The review details all aspects of pill properties including mode of action, metabolic effects and drug interactions based on over 530 references. From this review, in the final section entitled “Taking POPs effectively” the authors endorse that taking the pill every single day at the same time is essential[33]. In addition to the papers described above, the reference for this recommendation consists of two letters of personal correspondence and a textbook written for general practitioners to become more familiar with basic principles of birth control methods. Yet McCann’s guidelines serve both as the reference and the basis for similar recommendations found in Speroff and Darney’s A Clinical Guide for Contraception and Hatcher’s Contraceptive Technology[34,35].

Also contained within the progestin only literature, there is some suggestion that the type of progestin and the mode of delivery may affect the rate at which changes in cervical mucus
occur. Several old mini pill studies comparing different progestins, including many of which are either unavailable or no longer widely used, detail that there appear to be varying degrees of mucus response depending on the progestin[26,32]. The results of these studies are difficult to evaluate as they compare specimens in different women. Similarly, the topic of dose-dependent changes on mucus is essentially unexplored. As for route of administration, both Depo-Provera and the Levonorgestrel implant (Norplant) having been shown to cause more gradual declines in mucus quality after administration[36,37]. More recent evaluations of the LNG-IUS system has shown that users of the LNG-IUS have mucus that is substantially different from usual metrics and highly impenetrable by sperm compared to controls[8]. Moreover, temporal studies of LNG-IUS and cervical mucus indicate that mucus quality and sperm penetration is rapidly, but not universally altered following insertion[38].

It has been demonstrated that peak protein levels of one of the most important secreted protein in cervical mucus MUC5b, occurs at least one day after peak expression of mRNA levels[39]. Therefore there is interest in finding a regulating mechanism for “fast” mucus changes. Current research has focused on non-genomic estrogen receptors as well as the role of membrane channels in hydration[24,40–43]. In the case of progesterone, at least two classes of membrane associated progesterone receptors (MAPRs) have been described, including progesterone membrane component 1 and 2 (PGRMC1, PGRMC2) as well as membrane progestin receptors α and β (mPRs)[21]. Both PGRMC1 and mPRs have been demonstrated to serve functions in the endometrium and oviduct via non-nuclear protein cascades[44][45] (See Figure 1 in addendum). PGRMC1 for example has been shown to impair binding of oxytocin with its membrane receptor and that it mediates an anti-apoptotic effect in ovarian cells via non-nuclear protein cascades[44,46]. Although PGRMC1 expression was noted in Hela cells, which are derivative from a squamous cell cervical cancer line, neither PGRMCs nor mPRs have been identified in human mucus producing endocervical cells[47]. Initial steps towards exploring this possible source of regulation further would be to confirm the presence of non-nuclear progesterone receptors in these cells.
1.3 Design and Methodology

General summary of the approach:
This study will consist of two parts: (1) examining cervical mucus during a normal cycle (2) suppressing endogenous hormone production, adding back therapy through estrogen patches and randomizing women to assess the impact of estrogen withdrawal versus progestin administration on mucus. Serum, cervical mucus and endocervical brush samples will be collected at defined points throughout the experiment. Figures 2 and 3 below illustrate the experimental design.

Figure 2: Sampling Plan for Baseline cycle

Figure 3: Sampling and Hormone and Replacement Plan for Randomized Cycles

1.3.1 Subject Selection
A prospective cohort of healthy, normally menstruating women will be recruited. No subpopulations of women will be specifically included or excluded, and no special classes of subjects will be enrolled. For detailed inclusion/exclusion criteria see Table 1.

Table 1: Inclusion and Exclusion Criteria
Inclusion Criteria

- Normal menstrual cycles of 25-35 days in length for at least previous 3 cycles
- 21-40 years of age
- BMI >18, <35
- Serum P4 ≥ 3 ng/ml on single sample collected between days 18-25 of self-reported menstrual cycle
- Flexible schedule allowing blood draws on less than 48 hour notice
- In good general health
- Commit to using non-hormonal contraceptive methods without spermicide during study period except those prescribed in the experimental protocol
- No objections to taking study drugs
- No objections to refraining from intercourse the night before any sampling and willing to using condoms during vaginal intercourse.

Exclusion Criteria

- Oral contraceptive use or other hormone supplement within the preceding 2 months
- Women with current cervical infection
- Evidence of abnormal cervical cytology
- Use of any IUD for contraception
- Long-acting hormonal contraceptive use in the past 12 months (e.g., Depo-Provera®)
- Contraindications to study drugs
- Current or past pregnancy within the previous 6 months or currently trying to conceive
- Desiring to conceive in the next 8 months
- Breastfeeding in the past 2 months
- Diagnosed Diabetes or Metabolic Syndrome
- Diagnosed Polycystic Ovary Syndrome
- History of, or self-reported, substance abuse
- Smoker
- Previous infertility treatment excluding male factor issues
- Use of an investigational drug within the past 2 months
- History of excisional or ablative treatment procedure on cervix (ie. LEEP, Cryotherapy, Cold Knife Cone)
- Current treatment for a vaginal infection such as Bacteria Vaginosis
- History of Venous thromboembolism (VTE) or inherited thrombophilias

1.3.2 Treatment Cycles:

The study will begin with the first day of menstruation following enrollment. This cycle will be untreated but monitored and sampled (see figure 2 and section 1.3.3 for sample collection schedule). In order to determine points corresponding to late follicular and ovulation in these women, subjects will be provided with Clearblue® Ovulation Test (Inverness Medical Innovations, Inc., Waltham, Massachusetts). The fertility monitors test for urinary metabolites of LH, which will aid in staging of the menstrual cycle. At the end of the natural cycle treatment (menses), subjects will receive the gonadotropin releasing hormone (GnRH) agonist Lupron® (leuprolide acetate; Abbvie, Chicago, IL) in a 11.25 mg single injection which is sufficient for 3 months of continuous suppression. At 3 weeks, a visit will occur where an additional blood aliquot will be obtained and sent to the endocrine lab to confirm suppression. A phone call following confirmation will be made to instruct the subject to begin patches. We have previous experience with this model that shows ovarian suppression is reliably achieved at 21 days[48]. After confirmation of ovarian suppression and collection of baseline serum samples, all subjects will undergo replacement of endogenous estrogen with patches.

Transdermal E2 patches changed twice weekly (Vivelle-Dot®, Noven Pharmaceuticals Inc.; Miami, FL) will be used to replace E2, with a 0.1 mg/day transdermal dose having been previously shown to restore circulating E2 levels in ovarian-suppressed women to 89 ± 38
pg/ml[49], levels comparable to the late follicular and luteal phases of natural cycles[50,51]. Women will be kept at this level until prior to a randomized intervention at which point the day prior we will bring the women to mid-cycle levels (.3 mg/day). Levels of E₂ in normal women during the mid-cycle surge have been reported to be 335 ± 21 pg/ml [51] or approximately 3 times the E₂ concentrations measured with a 0.1 mg/day transdermal E₂ dose[49]. Pharmacokinetic data of E₂ patches demonstrates that half-life is approximately 5.9-7.7[52]. Again previous use of this replacement schedule demonstrates that these levels approximate serum hormone levels of normally cycling women[48]. In between interventions the subject will be maintained at the .1 mg /day level to prevent menopausal symptoms. At the end the subject will be placed on oral micronized P₄ (200 mg/day) for 5 days to trigger a withdrawal bleed. This dose causes circulating P₄ levels similar to those detected during the luteal phase of the menstrual cycle[56].

**Table 3: Sample Collection Schedule for Natural Cycles.**

<table>
<thead>
<tr>
<th>Experimental Cycle</th>
<th>Phase of Cycle</th>
<th>Days of Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Menstrual Cycle</td>
<td>Early Follicular</td>
<td>Days 3-5</td>
</tr>
<tr>
<td></td>
<td>Mid Follicular</td>
<td>Days 7-9</td>
</tr>
<tr>
<td></td>
<td>Late Follicular</td>
<td>Days 11-13</td>
</tr>
<tr>
<td></td>
<td>Ovulation</td>
<td>Days 0-1 Post LH-Surge (“Peak” fertility home test result)</td>
</tr>
<tr>
<td></td>
<td>Mid Luteal</td>
<td>Days 6-9 Post LH Surge</td>
</tr>
</tbody>
</table>

For the intervention phase women will be randomized to receive two different interventions twice to assess the effects of a single dose of oral progestin, Norethindrone (NET) versus estrogen withdrawal only. The dose of NET .35 mg is the same dose found in commercially sold oral progestin-only contraceptives[57]. During the intervention cycles, women will be started at the .1 mg dose. After at least 5 days the subject can undergo an intervention. The day prior to the intervention the subject will have her patches increased to the .3mg level. On the day of intervention that subject will either leave the patches on and take a NET pill or the subject will have the patches removed. At this point, in order to evaluate temporality of effect, we will collect several timed samples over 24 hours from initiation of intervention (see Figure 4). Following this 24-hour collection, all patients will be placed again on .1 mg patch for ~5 days as a washout. Recognizing there is some burden to allotting time to come to the visits, the subjects may schedule their next intervention a time beyond 5 days that is convenient for them. Again, we will only increase to the .3 mg the day before the scheduled intervention is to take place. All in all, the subject will receive 4 intervention/24 hours sampling periods, 2 of which will be the NET and 2 of which will be the estrogen withdrawal. See Figure 3 for replacement and collection schedule during this part of the experiment. Finally, after the progesterone withdrawal, subjects will be placed on a low dose oral contraceptive pill (.35 mcg ethinyl estradiol/1 mg norethindrone acetate) for management of side effect related to leuprolide.
1.3.3 Monitoring and evaluation

**Ovulation testing:** Subjects will be provided with Clearblue® Fertility Tests (Inverness Medical Innovations, Inc., Waltham, Massachusetts) for use beginning between days 8-10 of the menstrual cycle (depending on individual cycle length history) and continuing daily for up to 15 days or until the LH surge is detected. The device reports a positive result on detection of the LH surge which should occur approximately 24-36 hours prior to ovulation.

**Collection of samples:** At each visit where we are collecting data, blood samples and mucus samples will be collected. For blood samples, approximately 5 ml of blood will be collected and sent for analysis. A basic gynecological exam will be used to collect cervical mucus. An unlubricated speculum will be placed so that the cervix will be fully visualized. A large dry sterile swab used to clean the ectocervix of any debris. We will obtain fresh mucus using a special endocervical aspirator (Unimar Aspirette device, Cooper Surgical, Trumbull, CT, USA)[58]. Grasping ring forceps will be used for collecting thick or densely adherent...
endocervical mucus. For the days we are collecting endocervical cells for RNA analysis, following mucus collection, an endocervical brush will be inserted into the os and then immediately rinsed into a special RNA preserving reagent. Mucus will be transported in the collection device to a laboratory area close by where analysis will be conducted. Brush samples and any remaining mucus will immediately be frozen for later analysis.

Five collections will be performed during the natural cycle between days 3-5 (early follicular), 8-10 (mid follicular), 11-13 (late follicular), within 24 hours of positive ovulation test (ovulation), and days 6-9 post ovulation (mid luteal) (see Table 3).

Figure 3 shows the sample collection during the second part of experiments when we replace hormone exogenously. The patient will undergo 4 periods of sample collection each spanning a 24 hours period. The subject upon initiation of the randomized intervention (estradiol withdrawal vs. NET). Each collection period will include six samples (see figure 4). This sampling schedule will take place over 2 days. Beginning in the morning, we will collect samples at time 0 (initiation of intervention), 2, 4, and 6 hours. They will then return the next morning for collections at 22 and 24 hours. There will also be one sample collection at the beginning of the period (3 weeks after the Lupron shot), to confirm that ovarian suppression has been achieved and mucus is poor.

This means there will be 5 collections in the natural cycle and 4 (interventions) x 6 (collections/intervention) + 1 (prior to start of period) for a total of 30 collections. These will occur over 15 visits. Enrollment can also be combined with the first collection visit to make 14 total visits). Measure outcomes are further described below.
**Visits 8, 10, 12, and 14** will include serum hormone levels, cervical mucus scoring, sperm penetration testing at 0, 2, 4, 6 hours. Endocervical sampling will be done at 6 hours.

**Visits 9, 11, 13, and 15** will include serum hormone levels, cervical mucus scoring, sperm penetration testing at 22 and 24 hours (from previous day). Endocervical sampling will be done at 24 hours.

# You will be randomized to receive NET at 2 out of the 4 24 hr visits (visits 8, 10, 12, and 14).

@ Sperm Penetration Testing is an experiment done in a laboratory where donor sperm is placed in extracted cervical mucus and evaluated for their ability to swim.

Please note the protocol will have certain flexibility regarding initiation of certain parts of the study in order to minimize study burden on the subjects. Enrollment (visit 2) and the first collection (visit 3) can be combined for one longer visit if the subject desires. During both natural and artificial cycles, a personalized study schedule regarding hormone replacement plan and sampling dates will be given to the patient. A study diary will be kept by the patient recording all medications taken by the patient as well as the daily results of the home fertility test. Almost all sampling visits have a 3-day window during the natural cycle except days marking

<table>
<thead>
<tr>
<th>Study Month</th>
<th>Natural Cycle</th>
<th>Lupron Injection</th>
<th>Randomized Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corresponding Experimental Time</td>
<td>EF</td>
<td>MF</td>
<td>LF</td>
</tr>
<tr>
<td>Visit Number</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Consent</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical History</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Urine Pregnancy Test</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitals (Blood Pressure, Pulse)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height and Weight</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Progesterone+ Estrogen Levels</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cervical Mucus Scoring</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sperm Penetration Testing@</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Endocervical cell collection for RNA testing</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Receive Home Fertility Test Kit</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receive Study Diary</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receive Combined Oral Contraceptive (at end of visit)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupron or equivalent Injection</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receive Study Patches</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receive Progesterone Pills</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receive NET</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study wrap-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated time</td>
<td>90 m</td>
<td>30 m</td>
<td>15 m</td>
</tr>
</tbody>
</table>

* Visits 8, 10, 12, and 14 will include serum hormone levels, cervical mucus scoring, sperm penetration testing at 0, 2, 4, 6 hours. Endocervical sampling will be done at 6 hours.
** Visits 9, 11, 13, and 15 will include serum hormone levels, cervical mucus scoring, sperm penetration testing at 22 and 24 hours (from previous day). Endocervical sampling will be done at 24 hours.
# You will be randomized to receive NET at 2 out of the 4 24 hr visits (visits 8, 10, 12, and 14).
@ Sperm Penetration Testing is an experiment done in a laboratory where donor sperm is placed in extracted cervical mucus and evaluated for their ability to swim.
ovulation in the natural cycle. Similarly, the time between interventions is flexible as long as they are at least 5 days apart and the subject is able to come for back-to-back days of the 24-hour collections in the replacement cycle. Recognizing that the 24-hour samplings are time consuming and potentially burdensome, this gives flexibility to the subject on when these days occur. Also, as this is well within the three-month suppression period of leuprolide, offering this flexibility should not be a problem.

Moreover, cycles themselves can be initiated with some flexibility. Following leuprolide administration, the artificial cycle can begin between 21 to 28 days later allowing for subsequent visits to occur on weekdays or times best suited for the subject.

Cervical Mucus Testing: During and following the collection, the clinical measures of Mucus Scoring, Slide Testing and Penetration Testing will be evaluated as outlined by #1, #2 and #3 below. The remaining mucus as well the endocervical brush sample will be frozen for laboratory evaluations at later time (#4, #5, and #6). In order to prevent disruption of sperm-mucus penetration tests, sexually active women will be asked to use non-spermicidal condoms for vaginal intercourse. The women’s health research unit will provide such condoms to subjects.

1. Cervical Mucus Scoring

During and immediately following every mucus collection, these metrics will be graded: volume, consistency, cellularity, spinnbarkeit and ferning. This scoring system is described in the WHO laboratory Manual for the Examination and Processing of Human Semen and is a modification of the original Insler Score. Table 5 below outlines the components and grading of the score. A score of 10 is considered to be concepitive mucus favoring sperm penetration.

<table>
<thead>
<tr>
<th>Score:</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume- as estimated by amount that fills collection catheter</strong></td>
<td>0 ml</td>
<td>.01-.1 ml</td>
<td>.1-.29 ml</td>
<td>= &gt; .3 ml</td>
</tr>
<tr>
<td><strong>Viscosity</strong></td>
<td>thick, highly viscous, premenstrual mucus</td>
<td>mucus of intermediate viscosity</td>
<td>mildly viscous mucus</td>
<td>watery, minimally viscous, mucus</td>
</tr>
<tr>
<td><strong>Ferning</strong></td>
<td>no crystallization</td>
<td>atypical fern formation</td>
<td>primary and secondary stem ferning</td>
<td>tertiary and quaternary stem ferning</td>
</tr>
<tr>
<td><strong>Spinnbarkeit</strong></td>
<td>&lt;1cm</td>
<td>1–4cm</td>
<td>5–8cm</td>
<td>9cm or more</td>
</tr>
<tr>
<td><strong>Cellularity</strong></td>
<td>&gt;20 cells per HPF or &gt;1000 cells per Pl</td>
<td>11–20 cells per HPF or 501–1000 cells per Pl</td>
<td>1–10 cells per HPF or 1–500 cells per Pl</td>
<td>0 cells</td>
</tr>
</tbody>
</table>
2. Simplified Slide Test

For both the SST and sperm-cervical mucus penetration test, cryopreserved sperm derived from our sperm bank at OHSU will be unfrozen and confirmed for activation. Prethaw sperm count, motility and morphology sufficient for fertilization will be documented prior to use. Following thaw we will re-evaluate sperm count and motility.

A simplified slide test (SST) will be performed again according to the WHO Laboratory Manual. In this test, a drop of fresh cervical mucus is placed on a clear glass slide and flattened under a cover slip, surrounded by semen placed at each edge of the cover slip, incubated for 30 min at 37.1°C. Penetrability will be determined by the presence of any sperm <.5 cm the edge of the mucus sample. The outcome is graded as having normal penetrability, poor penetrability or abnormal penetrability (penetration with immotile sperm). The simplified slide test (SST) was initially designed for use only when scant or thick mucus prohibited performance of the sperm-cervical mucus penetration test (SCMPT) described below. We will perform this test if enough mucus is available for two different penetration tests.

3. Sperm-cervical mucus penetration test

Sperm penetrability will also be assessed using the sperm-cervical mucus penetration test (SCMPT). The SCMPT is an in vitro sperm penetration test, described in detail by Eggert-Kruse[59]. Fresh cervical mucus is immediately aspirated into a capillary tube. It is then sealed from one end— and with a single drop of mucus protruding from the open end— it is placed into a reservoir of semen. Migration distance of the foremost spermatozoon, density of penetration using an average of the total number of live sperm at each centimeter between 1 and 5 cm, and the quality of motility at the distal end of the capillary tube are assessed (see table 5 below). The test will be performed once, at 2 hours after incubation. Each parameter is graded on a scale from 0 to 3 for a total score of 9. Scores greater than >= 6 are considered to have good penetration[60]. For both sperm-penetrations tests these tests will be done by 5 days following mucus collection in accordance with recommendations of the WHO manual.

Table 6: Sperm Penetration Meter Score as adapted from Eggert Kruse [59]

<table>
<thead>
<tr>
<th>Penetration Parameter</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration Distance (mm)</td>
<td>&lt;15</td>
<td>15-29</td>
<td>30-44</td>
<td>&gt;=45</td>
</tr>
<tr>
<td>Sperm Density</td>
<td>0-9</td>
<td>10-49</td>
<td>50-99</td>
<td>&gt;=100</td>
</tr>
</tbody>
</table>
Motility Grade | Immotile | In Situ motility (shaking) | 50-99 | >=100 |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slow forward motility</td>
<td>Highly Propulsive Motility</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Real-time PCR of MAPRs.

Frozen samples of rinsed endocervical brushings in RNAlater (Qiagen) will be thawed for quantitative real-time PCR. After total RNA is isolated and purified, it will be reverse transcribed into cDNA using primers. Primers for PGRMC1 and PGRMC2, mPR α and β, and PGR will be created using Primer Express software [Applied Biosystems (ABI), Foster City, CA, USA]. The primer and probe sequences for PGRMC1 and PGRMC2 have been validated recently in the macaque ovary by comparing the values obtained from real-time PCR with microarray data[61]. Transcript levels in each sample will be quantified against a five-point standard curve. Samples from similar points in the cycle will be pooled. The real-time PCRs will be performed in MicroAmp Fast Optical 96-well plates on an ABI 7500 Fast Real-time PCR System.

5. Mucus Rheological Characteristics

Once mucus samples have been evaluated by mucus score and sperm penetration (#2 and #3 above), the remaining, de-identified samples will be sent to the Complex Fluids and Soft Solids Laboratory at Oregon State University. OSU will only receive samples that are de-identified and coded by the subject number so that their tests and analysis can be correlated to serum hormone levels and results from #2-3 above. Labels on the samples will contain the subject number, the sample number (visit number) and the date the sample was originally obtained. The data returned would both be aggregated data of samples pooled from similar time points as well as data sorted by subject in order to account for inter-subject variability. OSU will use the same coding system as OHSU (subject number).

Led by Dr. Travis Walker, the lab specializes in developing both theoretical and experimental methods to study complex fluids, soft solids, miscible fluid interactions, and biological systems. They will use both sheer and extensional rheological techniques to provide detailed mechanical characterization of the cervical mucus samples. These will be performed on samples that are otherwise destroyed and will be destroyed following testing. This mechanical data will be correlated to conventional mucus scores.

6. Ion Content

Following mucus collection, an aliquot of the coded mucus may be sent to the OHSU elemental analysis core lab to measure ion content of mucus. The lab will only receive samples that are coded by the subject number so that their tests and analysis can be correlated to serum hormone levels and results from #2-3 above. Labels on the samples will contain the subject number, the sample number (visit number) and the date the sample was originally obtained. The elemental analysis core lab is an on campus facility run by Martina Ralle Ph.D. that provides quantification of ions such as sodium, calcium in biological
16 samples. Read out will be used to help understand changes in mucus composition due to hormonal fluctuations.

1.3.3 Subject Recruitment and Compensation

We will recruit subjects from the Portland metro area. Most of our enrollment will likely be in response to notifications of research opportunities posted on campus (print, flyers, and online), and through community sources soliciting volunteers and study participants (e.g., Craig’s List). Subjects will be reimbursed $45 per collection and $250 for the leuprolide acetate injection. In the artificial cycles, we will give a 100 dollar bonus for completing each 24 cycle in order to additionally compensate subjects for the time spent at OHSU between 2 –hour collections. Total compensation for completion of all aspects of the study will be $2000.

1.3.4 Description of drugs and devices to be studied

GnRH agonist: Lupron (leuprolide acetate) is FDA-approved for use in men for the palliative treatment of advanced prostate cancer. Lupron belongs to the GnRH agonist class of drugs. In addition to prostate cancer, Lupron is approved for use in women for the management of endometriosis and for fibroid surgery to improve anemia resulting from vaginal bleeding due to fibroids. Lupron is also approved for use in children for the treatment of central precocious puberty[62]. Our proposed use of Lupron is unapproved because we plan to use it in healthy women, which represents a different patient population. Our reason for using it in a different patient population is not to support FDA approval for a new indication, but is based on purely scientific reasons. As this is a small prospective study, to be able to study the outcomes in a crossover fashion increases the statistical power and reliability of results.

Using Lupron will allow us to determine what happens to cervical mucus when the menstrual cycle is stopped in young women thus mimicking a menopausal state, and what happens when E₂, P₄ and NET are added back in the absence of ovarian processes (i.e. follicle growth, corpus luteum development and function). Lupron act by down regulating GnRH receptors in the pituitary, thus inhibiting gonadotropin release. This mechanism of action is the same in everyone receiving leuprolide acetate, so the risks of its use in women with endometriosis or fibroids are a good surrogate for the expected risks of its use in healthy women. The dose of Lupron selected (11.25 mg) is the FDA approved 3-month dose used in women. Moreover, higher doses of leuprolide acetate are used safely (up to 30 mg for 3 month formulation). Thus, the use of Lupron is not expected to significantly increase the risks to subjects.

Estradiol: Transdermal E₂ doses were selected to in order to reproduce circulating E₂ levels consistent with a normal menstrual cycle and not to maintain replacement levels of hormone. We are assuming that absorption of E₂ from multiple patches will be linear. The form of E₂ in the patches is identical to endogenous E₂ from the ovaries, and the transdermal route of administration is preferable as it avoids the first pass effect in the liver and thus is more physiologic than oral administration[49].

The transdermal E₂ patches will be used at higher than United States Food and Drug Administration (FDA)-approved doses during the .3 mg patch days. However, subjects will be at this level for a short period of time. The amount of E₂ delivered at the highest dose (0.3 mg/day on days 7-9, 28-30) is within the normal physiologic range of E₂ secreted by the ovary during the menstrual cycle (0.07-0.5 mg/day, Vivelle-Dot package insert), so it is anticipated that even at the highest doses subjects will not be exposed to circulating E₂ levels that exceed what they are routinely exposed to during their menstrual cycle. Because the form of E₂ in the patches is identical to natural E₂, the route of administration allows for physiologic E₂ delivery, the subjects will only
be acutely exposed to unapproved doses, and our doses are anticipated to cause circulating $E_2$ levels similar to what the subjects are routinely exposed to during their menstrual cycle, we do not anticipate a significant increase in risk to the subjects by our proposed unapproved use of the transdermal $E_2$ patches. In fact, this replacement schedule was used in a previous study and $E_2$ peak levels were closely aligned to natural cycle peaks (mean $E_2$ levels 210 pg/ml in artificial vs 230 pg/ml in natural cycles)[48].

Progestins: Progesterone is widely used in treatment of preterm labor as well as in support of in vitro fertilization cycles. We chose oral micronized progesterone to maintain the luteal phase as this is more tolerable than daily IM injections to patients. Approximately 200 mg of oral micronized progesterone is needed to reach mid luteal phase levels in premenopausal women[63]. Studies have been conducted with progesterone at much higher doses (300, 600, 1200 mg oral micronized) and no serious adverse effects were found at these higher levels[65].

Norethindrone (NET) is one of the most widely studied progestins among contraceptive steroid hormones with no associated serious adverse events and no upper dose limits known to cause toxicity[66,67]. Metabolic studies on NET alone have shown negligible effects on metabolic measures such as lipid levels, carbohydrate metabolism. They have been shown to have little or no effect on coagulation factors and when used without estrogen does not increase the risk of VTE. Norethindrone and its acetate ester derivative norethindrone acetate are often packaged with Ethinyl Estradiol in common oral contraceptive formulations. These “first generation” pills are some of the oldest FDA approved combined oral contraceptive pill formulations with decades of population data. VTE risk represents the most common serious complication of oral contraceptive pills with a 3 to 4 fold increase in the baseline VTE risk of 3-4/10,000 in oral contraceptive users[68].

1.3.5 Admission Procedure

Potential study subjects will undergo a telephone screen by the study PI or the OHSU Women’s Health Research Unit’s (WHRU) recruitment specialist. All study procedures will be performed in the WHRU outpatient Clinic. Following confirmation of study eligibility and informed written consent by either PI or study staff, confirmation of ovulatory status by serum progesterone level during days 18-25 of their menstrual cycle. The OHSU research pharmacy will be utilized for any and all drug dispensing during the study.

A computer-generated algorithm through the OHSU research pharmacy will perform randomization. Treatment allocation and blinding will occur at the end of the artificial study and prior to the start of the randomized cycle (visit 17). Blinding will single blinded so that the researcher collecting the mucus and performing the mucus scoring and sperm penetration tests does not know the patient status. The patient will know there their status and will be given strict instructions to not inform the researcher of their allocation group during this part of the study. We will ask study patches during this time to be worn during non-visible areas. We chose this design because we are unable to provide placebo estrogen patches. However, we feel that this single-blinding design is acceptable, as patient knowledge of their status should not affect mucus scores or serum hormone levels.

1.3.6 Follow-up procedure

All data for this study will be collected during scheduled visits to the Women’s Health Research Unit. (see study flow figure). Our research unit has been highly successful in recruitment, enrollment and retention of subjects as part of our screening process, ease in scheduling, and appropriate compensation for study procedures.

1.3.7 Criteria for discontinuation
Patients have the right to withdraw from the trial at any time on their own request for any reason; the reason for withdrawal will be recorded in detail. No further data will be obtained from the patient once withdrawal occurs. Any data obtained up to this point will be analyzed in accordance with intention to treat.

The Data and Safety Monitoring Board (DSMB) for the WHRU at OHSU will meet on an as needed basis if any serious adverse events related to the study protocol are reported by the investigator.

1.3.8 Data Collection

See Section 1.3.3 for detailed descriptions of our planned analysis of mucus samples. Additionally, serums samples will be sent for laboratory testing of LH, FSH, E$_2$ and P$_4$ levels immediately following collection. As mentioned above, endocervical brush samples will be frozen for later usage. These will be stored in a repository freezer in the Women’s Health Research Unit at OHSU.

1.3.9 Data management

The PI will manage data collection and monitoring for this study. Study personnel and study investigators will collect data at the procedure visit.

All patient data sheets will be kept confidential and in a locked office. A unique patient study identification number will be placed on the data forms. Study identifiers kept in a logbook will be assigned to each patient to protect confidentiality. Subjects will be contacted by the reminder method of their choice one-day prior to each collection by study coordinators or the PI. No patient names will be included with the study data during statistical analysis. Forms will be transferred to the PI’s locked office for data entry and analysis. Patient identifiers will be stored separately from the data files on the PI’s password-protected computer. Only the study investigators will have access to this identifier list.

Sample data will be analyzed with STATA. Once the data has been entered into STATA, descriptive statistics will be used to check to outliers. In addition, random data entry checks will be performed to further verify correct entry of the data.

1.3.10 Data analysis

Data will be extracted and entered into STATA on a password-protected computer. Analysis will be performed with the blinded data.

The primary analytic approach will be to compare differences in fertile cervical mucus (WHO score $\geq$10) and penetrable cervical mucus (SST, SCMPT $>6$) between the two groups. Frequencies will compared using McNemar’s test and median scores can be compared with a Wilcoxon Signed-Rank tests. A Cochran-Mantel-Haenszel statistic can be used to examine the effects of blood in the cervical mucus sample on test results. Real-time PCR data will be log-transformed and then subjected to one-way analysis of variance using the mixed model subroutine in SAS software.

1.3.11 Number of subjects and statistical power

This is meant to be a small pilot study and as such, a power analysis was not performed. Absolute quantification is less important than relative measures. A number of ten was chosen for the study because this will provide some buffer against biological variability as well as an
estimated 70% completion rate. This sample size collection is consistent with design of similar experiments exploring biological mechanism.

We currently have enrolled 7 subjects. However, an interim analysis noted that the original protocol did not optimize patch hormone levels immediately prior to the 24-hour collection and that we did not need more data from the original “middle artificial cycle”. The data most critical to our clinical experiment is the randomized cycles and the study has been amended to optimize data collection for these points in fewer visits.

The 7 subjects enrolled under the original protocol (approved by the IRB 19 March 2015) will complete the study under that protocol and will not be affected by these changes. The remaining subjects enrolled will follow this updated protocol.

1.3.12 Study limitations

**Clinical Relevance of Outcomes:**
Currently the clinical measure we are using (WHO Mucus Score, Sperm Penetration Testing), have been validated as measures of fertility. At this time, only associations between the actual scores and contraceptivity have been made in other experiments[8,59]. This is one of the most important limitations of research in cervical mucus. Studies that prove if something is contraceptive require large numbers and examination of hard outcomes like pregnancy. Meanwhile our study employs smaller numbers in order to do the detailed testing that can give us mechanistic insight. While our data can give suggestions into contraceptive efficacy as we are performing tests that look at sperm penetration, it cannot be used to accurately estimate efficacy in real-life use of progestin contraceptives.

The addition of Dr. Walker’s rheological testing will provide additional mechanical information on mucus samples that will otherwise be destroyed. His lab is well equipped to conduct a suite of molecular, rheological, surface, and thermal characterizations of various complex fluids and soft solids. This preliminary data can hopefully be used to find a better way of evaluating mucus. This would aid in contraceptive studies

**Experimental Conditions and Generalizability:**
The experiment has been designed such that natural HPO influences are suppressed. The rigid control of hormonal conditions allows us to better discriminate between estrogen and progesterone effects on mucus in a novel way, providing insight into its regulation. These are non-physiologic conditions and our results are not generalizable to the actual physiology that occurs in normal cycling women.

**Sampling Frequency:**
Timing of effect is an important outcome of this experiment. Once estrogen is withdrawn and progesterone or a progestin is administered, we would like to estimate the temporal nature of this effect. However, our discriminatory abilities will be limited by sampling frequency that is both reasonable for subject convenience as well unlikely to influence the outcomes of subsequent samples. Based on previous data, we believe that two hours is the minimum spacing needed to give us meaningful temporal data and which also minimizes previous mentioned considerations.

1.3.13 Duration of project

The study will begin once OHSU Institutional Review Board approval has been obtained (estimated time for approval: 8 weeks). Given small enrollment numbers and our history of enrollment for experimental trials, we anticipate 10 months to complete enrollment. Following
sample collection, initial clinical data analysis can be performed and report writing can be completed within four months. For testing of frozen samples for PGMC1, and PGMC2 an additional 3 month of time will be used to conduct laboratory experiments.

1.4 Project management

This project requires collaboration between the Women’s Health Research Unit, the investigators, and the study personnel. The study will remain the responsibility of Dr. Leo Han. Drs. Jeff Jensen, Ov Slayden and Alison Edelman will be the faculty mentors for the project.

1.5 Links with other projects

This study is not linked to other projects.

1.6 Main problems anticipated

Full sample collection: This study contains a rigorous sample collection timeline over a short period of time. Subjects will be thoroughly briefed to the extent of their involvement during the informed consent process. Furthermore, compensation will be structured such that there will be an incentive to come for all collection appointments.

Interpersonal Variation: One of the difficulties in studying cervical mucus is the person-to-person variation in normal secreted mucus quality and quantity. Moreover ovulatory cycles vary in women and can affect results if the exact point in the cycle is unknown. For this reason we have chosen this cross-over study design, a comparison of serial samples of mucus in the same woman under different conditions. Through this design a woman can serve as her own controls. In addition to this concern, we are unsure if there is a time-effect after administration of leuprolide and a randomized crossover design will account for this. Last there is some concern that when we administer P₄ or NET, the samples maybe inadequate for running all of the tests we wish to conduct. Previous studies have managed the issue of inability to obtain samples by assigning a “0” value to both mucus scoring and sperm penetration tests[36,37] or by excluding them from the calculations[8]. However, we will have additional outcomes for comparison, as we will also be assessing temporal changes in addition to qualitative changes.

1.7 Expected outcomes of the study and dissemination of findings

In all groups, we expect that estrogen priming will lead to fertile mucus. However, based on previous research, we anticipate evidence of direct progesterone effects either qualitatively or temporally. Currently, there is suggestion that mucus changes could occur as soon as 2 hours after administration with a return to fertile mucus in equally rapid fashion. Based on our reading, there is some skepticism to the validity of this data and our findings could challenge these previous reports. Additionally, it would add to the overall paucity of data that assesses the timing of onset and offset of progesterone action on cervical mucus. This information has important implications in terms of our usage of progestin based birth control methods and perhaps would necessitate a re-evaluation of our current clinical recommendations for use of progestin only contraception. We may also however find qualitative differences between estrogen withdrawal effects and progestin mediated effects. It is possible that the progestin influences are related primarily to mucus hydration and not mucin protein production. In this case we may see
differences in the components of mucus scores or sperm penetrability without outright differences in favorability.

Again, consistent with our overall hypothesis of mechanism, we expect that evidence of MAPRs in endocervical cells will be found and this would be a novel finding. MAPRs would then serve as a regulatory mechanism from which further pathway elucidation could be elaborated. It also would offer a potential non-genomic target for drug design.

Some of the results of this study will be submitted for publication in one of the obstetrics and gynecology specific journals. Our data on MAPRs maybe more appropriate for a basic science journal in reproductive science.

1.8 References


[67] Stanczyk FZ. All progestins are not created equal. Steroids 2003;68:879–90.


Figure 1: Summary of signal transduction pathways active by progestins through mPR alpha and PGRMC1 (taken from Thomas, P)[69]