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The postcoital test in the development of new vaginal contraceptives†

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Abstract

Postcoital tests (PCTs) have been used for over a century in the clinical evaluation of infertile couples, and for nearly 70 years in the evaluation of new vaginal contraceptive products. PCTs have been largely replaced by more modern methods in the study of infertility, but they remain the most useful way to obtain preliminary data on the effectiveness of vaginal contraceptive products. The World Health Organization has described important aspects of the procedure. It involves collection of cervical mucus at a certain time point after intercourse and the counting and characterization of sperm found in the mucus. A wide range of progressively motile sperm (PMS) has been associated with pregnancy rates in infertility studies. Eligibility for contraceptive trials includes the requirement that couples achieve a certain threshold number of PMS per high power field at midcycle in a baseline cycle without the test product. The primary endpoint, or definition of a satisfactory result in test cycles, is predefined. A literature review identified 10 PCT studies of vaginal contraceptives involving nine test products. Phase II trials of vaginal contraceptives have not been deemed feasible in the development of any vaginal contraceptive to date. A PCT study of a test product can be predictive of contraceptive efficacy, although ultimate contraceptive effectiveness is influenced by the ease of use of the product, along with patient compliance. PCT results similar to results seen with products that later showed satisfactory performance in efficacy trials is the best indicator of likely success of a test product.

Summary Sentence

A product that performs well in a postcoital test (PCT) study goes on to demonstrate a high level of contraceptive effectiveness, although the PCT is not predictive of exact effectiveness; ultimate contraceptive effectiveness is influenced by the ease and convenience of use of the product.

Key words: vaginal contraception, vaginal ring or device, diaphragm, ferrous gluconate (iron), postcoital test, nonhormonal, monthly, non-coital, sperm motility and transport.

Introduction

The postcoital test (PCT) was first described by Marion Sims in 1866 [1, 2]. He examined a patient a few minutes after she had intercourse with her husband and observed active sperm in her cervical mucus. He later used the PCT as a means of evaluating the cause of infertility in eight women [3]. The procedure was further developed by Hühner in 1913 [4], and the Sims-Hühner test to determine whether "cervical factors" played a role in infertility

became standard practice in the evaluation of infertile couples fo r many decades.

The World Health Organization, in its publication *WHO Laboratory Manual for the Examination and Processing of Human Sperm*,[1] states that the aims of a PCT are to "determine the number of active spermatozoa in the cervical mucus and to evaluate sperm survival and sperm behaviour some hours after coitus." The manual describes important aspects of the procedure as shown in Table 1. It can be seen that small changes were made between the fourth and fifth editions [5, 6] and the manual has been interpreted in different ways, depending on whether it is being applied to infertility evaluation or contraceptive research.

Use of the PCT in the evaluation of infertile couples

As evidenced by changes in the WHO manual, standardization of the procedure was always a work in progress and many practitioners carried it out as they best saw fit. It was reported in 1995 that the PCT was used in 92% of obstetrics/gynecology departments with large fertility clinics in 16 European countries, but despite the existence of the WHO manual, there were "large differences in timing in relation to cycle and coitus, methodology used for the test, cut-off level of normality and treatments applied for abnormal test results." [7].

Variations in PCT for infertility

A review of research studies involving PCTs done in infertile women reveals the following variations:

- Length of time between coitus and collection of mucus for evaluation:
  This has been variously reported as being set at, for example, 2.5, 2–8, 6–8, 8–16, and 15–20 h [8–12].
- Method to determine when in the menstrual cycle to conduct the test:
  Dates of previous menses and basal body temperature charting were the methods used by most researchers [9–20]. Some later studies required a plasma progesterone level of >3 ng/ml during the luteal phase to give retrospective evidence of ovulation [8, 12]. Urinary luteinizing hormone (LH) dipsticks and ultrasound were rarely used [15, 17, 18, 20].
- Scoring of cervical mucus to assess time in cycle:
  Some of the five criteria cited in the WHO manual were used in most cases [8, 9, 11–13, 18, 21], but all five were used only in later studies [16, 20]. Not all studies required a cervical mucus score of at least 10 to be considered valid, although some required repeat of negative tests [13–15].
- Length of male abstinence
  While some studies recommend that the couple abstain from sex for 2–3 days before the PCT sex act [10, 14, 15, 20], proscription against the male partner masturbating is rare.
- Method of counting sperm
  Studies vary in the method of slide preparation (at least one investigator added saline to the vaginal preparation [22]), magnification used (most used, 400× [9, 10, 13, 14, 18]), number of fields examined (e.g., at least 3 [13], at least 5 [14], 10 [9]), and categorization of motility (e.g., 0–3 [10, 15, 17], not progressively motile vs. progressively motile [13, 14, 16, 19]).

The interpretation of the PCT—i.e., what may be considered a "normal" result associated with a higher risk of pregnancy—has also varied widely. The WHO manual fifth edition states: "The presence of any spermatozoa with progressive motility in endocervical mucus 9–14 hours after intercourse argues against significant cervical factors, and sperm autoimmunity in the male or female, as possible causes of infertility." However, a 1973 WHO publication stated "10 or more sperm/HPF [high power field] with directional motility may be considered satisfactory. Fewer than 5/HPF, especially when associated with sluggish or circular motion is an indication of oligoasthenospermia or abnormal cervical mucus." [23].

PCT prediction of pregnancy in infertility

A number of studies have been conducted in an effort to determine how well the PCT predicts subsequent pregnancy, with varying results. Using the WHO definition of any spermatozoa with progressive motility, some researchers found that a single sperm seen on the PCT was associated with an increased chance of pregnancy. Hull studied 80 women with at least 12 months infertility and found a five-fold higher pregnancy rate when at least one sperm with forward progression was seen in at least three HPFs in the cervical mucus 6–18 h after coitus [13]. Glazier studied 318 infertile couples and found that the ratio of pregnancy within the subsequent 18 months in those with at least one forward-moving sperm in each of five HPFs examined vs. those with no forward-moving sperm was 3.73 [14]. In a 2000 reanalysis of the same data, Glazener reported "the relative chance of conception in couples with a negative PCT was about a quarter of that when the PCT was positive." [24]. Similarly, Snick studied 726 infertile women and defined an abnormal result as the presence of at most one forward-moving sperm in the entire mucus sample [12]. Having this type of abnormal PCT was associated with a relative risk of live birth of 0.26. Dunphy found that among 94 infertile couples, those with at least one sperm per HPF showing at least sluggish motility had nearly five times the chance of conceiving compared with those with sperm with only in situ motility or no motility [15]. Eimers found that among 996 infertile patients, those with more than one progressively motile sperm (PMS) in the entire mucus sample had a 330% chance of conception relative to women with no sperm [16]. Similarly, Hessel found that the presence of one or more progressive forward-moving spermatozoa per HPF among 1624 newly referred infertile women was associated with spontaneous (meaning achieved without medical intervention) and overall ongoing pregnancy rates after 3 years of 37.7 and 77.5% compared with 26.9 and 68.8% after a negative test ($P < 0.001$) [25].

While it seems clear (and somewhat predicable) that having some sperm rather than no sperm in the cervical mucus is associated with a higher chance of subsequent pregnancy, it is more difficult to assess the likelihood of pregnancy associated with different quantities of sperm among women who have more than one sperm/HPF. Collins found that among 355 infertile couples, the pregnancy rate at 24 months was significantly higher in couples with at least five motile sperm/HPF vs. those with fewer (46.9 vs. 31.6% $P = 0.05$) [21]. Jette

[1] The first edition of the WHO manual was published in 1980, with second, third, fourth, and fifth editions published in 1987, 1992, 1999, and 2010, respectively. With publication of the fifth edition, the title changed from *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction* to *WHO Laboratory Manual for the Examination and Processing of Human Semen*. The fourth and fifth editions are available online [5, 6].

Table 1. PCT procedures, *WHO Laboratory Manual for the Examination and Processing of Human Sperm*

<table>
<tr><td></td><td>WHO 5th edition [6]</td><td>WHO 4th edition [5]</td></tr>
<tr><td>Length of time between coitus and collection of mucus for evaluation</td><td>“It is important that the mucus is evaluated in the laboratory at a standard time—between 9 and 14 hours after coitus” and “Some 2–3 hours after coitus there is a large accumulation of spermatozoa in the lower part of the cervical canal.”</td><td>“It is important for all laboratories to evaluate the mucus at a standard time after coitus. This time should be from 9 to 24 hours.”</td></tr>
<tr><td>Method to determine when in the menstrual cycle to conduct the test</td><td>The test should be done “as close as possible to, but before, the time of ovulation, as determined by clinical criteria, e.g. usual cycle length, basal body temperature, cervical mucus changes, vaginal cytology, serum or urinary luteinizing hormone or estrogen assays, and ovarian ultrasound examination.”</td><td>The test should be done “prior to ovulation and as closely as possible to the time of ovulation as determined by clinical criteria, i.e., usual cycle length, basal body temperature, cervical mucus changes, vaginal cytology, and, when available, serum or urinary oestrogen assays and an ovarian ultrasound examination.”</td></tr>
<tr><td>Method of scoring cervical mucus changes</td><td colspan="2">The 4th and 5th editions agree on this. Scoring is based on 5 criteria, each of which receives a score of 0-3 as shown below: mucus volume, viscosity, ferning seen on a dried slide specimen (an indicator of estrogen status), spinnbarkeit (stretchiness), and cellularity. A perfect score is 15. A score of 10 or above is considered indicative of midcycle cervical mucus most conducive to sperm movement.
<table>
<tr><th>Score</th><th>0</th><th>1</th><th>2</th><th>3</th></tr>
<tr><td>Volume (length of mucus in aspirette)</td><td>None</td><td>0.2-1.9 cm</td><td>2.0 - 5.5 cm</td><td></td></tr>
<tr><td>Viscosity</td><td>Thick, highly viscous, premenstru</td><td>Mucus of intermedia te viscosity</td><td>Mildly viscous mucus</td><td>Watery, minimally viscous,</td></tr>
<tr><td>Spinnbarkeit</td><td>< 1 cm</td><td>1 - 4 cm</td><td>5 - 8 cm</td><td>9 cm+</td></tr>
<tr><td>Fern Pattern</td><td>No crystallization</td><td>Atypical fern formation</td><td>Primary and secondary stem ferning</td><td>Tertiary and quaterna</td></tr>
<tr><td>Cellularity (using leukocytes and other cells)</td><td>>20 cells per HPF or >1000 cells per μL</td><td>11–20 cells per HPF or 501–1000 cells per μL</td><td>1–10 cells per HPF or 1–500 cells per μL</td><td>0 cells</td></tr>
</table></td></tr>
</table>

(*continued*)

Table 1. Continued

Length of male abstinence	"Couples should be instructed to abstain from intercourse, and the man from masturbation, for 2 days before the test"	Couples should "abstain from intercourse for at least 2 days before the test."
Method of counting sperm	No specification is made for the number of high power fields (HPFs) to examine. "A simple system for grading motility is recommended that distinguishes spermatozoa with progressive or non-progressive motility from those that are immotile." "The motility of each spermatozoon is graded as follows: o Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed. o Non-progressive motility (NP): all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed. o Immotility (IM): no movement."	i. "At least five microscopic fields are assessed in a systematic way to classify 200 spermatozoa. The motility of each spermatozoon is graded 'a', 'b', 'c', or 'd', according to whether it shows: o a - rapid progressive motility o b - slow or sluggish progressive motility o c - nonprogressive o d - immotility."

found a statistically significant increase in pregnancy rates among 205 infertile patients in those who had >20 motile sperm/HPF [11]. And Moghissi found an average of 16.8 sperm in the endocervix of 58 infertile women who became pregnant compared with 7.1 in 143 women who did not [26]. In a review article, Blasco stated that between the two extremes of >10 sperm/HPF with 50% having purposeful motility and <5 with >50% that do not move, the prognostic value of the PCT is limited [27].

An important factor to consider is the population from which participants in these studies were drawn. For obvious reasons, since the PCT was being used to evaluate infertility, these were generally populations of women being seen for infertility. However, infertility has many causes besides those involving sperm–mucus interaction. So even if certain causes were ruled out before the PCT, such as anovulation or tubal occlusion, there may have been other unidentified causes preventing pregnancy. The failure to conceive despite the presence of sperm on a PCT does not necessarily invalidate the test—it may indicate that a problem other than one involving sperm–mucus interaction is likely the chief cause.

PCT prediction of pregnancy in fertile couples

A better test of the PCT as a predictor of fertility would be a study done in women of proven recent fertility with the same partner, in whom good mucus is seen, and who engage in no other coital acts in that cycle. However, even in this population, important factors must be considered, length of time of follow-up being probably the most important. In any population of women attempting pregnancy, the pregnancy rate falls over time since the most fertile women achieve pregnancy first. This phenomenon affects the Pearl pregnancy rate that is being replaced by the life table analysis that provides the pregnancy rate for each month of follow-up and can provide a pregnancy rate for any length of follow-up.

To date, no perfect study has been carried out. Giner allowed only one coital act per cycle, and that act was studied in a PCT [9]. He did not find a correlation between pregnancy and sperm number or motility in the PCT, but the study was done in a population of women who had experienced recurrent spontaneous abortions, likely due to reasons other than problems with sperm/cervical mucus interactions.

Beltsos studied 200 couples who had discontinued contraception to become pregnant up to 3 months earlier [17]. They had no

history of infertility and no known risk factors for infertility or recurrent pregnancy loss. They underwent monthly PCTs based on their menses dates with daily urines collected for retrospective urinary LH testing in the lab (presumably because home LH test kits did not yet exist). Pregnancy occurred in 163 couples within 12 months. The PCT values for each woman were averaged and there was a small, but significant, difference in the number of sperm with purposeful forward motility per HPF among the women who became pregnant vs. those who did not (2.5 vs. 1.4, $P = 0.03$). However, 42% of cycles were found to have been mistimed and results were not recalculated using only correctly timed cycles.

Decline of PCT for infertility evaluation

In 1990, Griffith and Grimes published a review of the PCT and concluded that the PCT "lacks validity as a test for infertility." [28]. And in 1998, Oei published a paper showing that use of the PCT among infertile women did not affect pregnancy rate [18]. However, both papers were heavily criticized because, among other things, the couples had varying lengths of follow-up, there may have been other causes for infertility, and the PCT was not used to determine treatment [19, 24, 29].

In a comprehensive 2002 review of methods used to predict conception, Glazener concluded "in a population of infertile couples with otherwise normal results after complete investigations, the chance of conception could be predicted by their duration of infertility at first presentation and the result of the PCT, but not by semen parameters or the woman's age." [29]. Nevertheless, the PCT was gradually replaced in the infertility work-up by more modern tests and procedures, and the wide use of in vitro fertilization, which bypasses the cervical mucus–sperm interaction.

Use of the PCT in the evaluation of new vaginal contraceptives

Historical Use of PCT for development of vaginal contraceptive agents

However, the PCT continues to be used in the evaluation of vaginal contraceptives, both chemical products (e.g., spermicides) and mechanical barriers (e.g., diaphragms), as recently as 2017 [30]. The first published report of the PCT used in the evaluation of a contraceptive was a 1953 study of an experimental contraceptive jelly ("Jelly P") [22]. In it, 289 PCTs were done in 158 mostly postnatal women 2–72 h post coitus. Spinnbarkeit and time since last menses were used to estimate time in cycle. Motile sperm were found in six (2.1%) of the PCTs. In three cases, product use instructions had not been followed correctly. There were seven pregnancies among the 83 women who were followed for 3 months, yielding a pregnancy rate of 17.5 per 100 woman-years. Correlation between PCT and pregnancy was not attempted, but the authors concluded that they were "satisfied that the PCT is accurate and should be utilized more widely in the evaluation of spermicidal preparations used as contraceptives."

The PCT has subsequently been used to evaluate other vaginal chemical barriers, i.e., Advantage 24 gel [31], benzalkonium chloride (BZK) films [32], nonoxynol-9 (N-9) films [33], C31G gel [34], and ACIDFORM (later Amphora and Phexxi) gel [35], as well as several mechanical vaginal barriers, i.e., Lea's Shield [36], FemCap [37], Ovaprene vaginal ring [38], and the SILCS (later Caya) diaphragm [30, 39]. A review of the literature since 1953 identified 10 PCT studies of vaginal contraceptives involving these 9 test products and 3 control products (Ortho diaphragm [36, 37], Vaginal Contraceptive Film (VCF) N-9 film [32, 33], and Conceptrol N-9 gel [31]). They are summarized in the first three columns of Table 2.

Similarities in PCTs for vaginal contraception testing

Unlike PCTs done to evaluate infertility, these trials were done with similar methodology in most respects. Similarities in these studies are as follows:

- Inclusion criteria:
  Female participants had to have regular menstrual cycles, be protected from pregnancy by female tubal sterilization, and have no history of infertility involving themselves or their partner.
- Pre-PCT activities:
  With the exception of the Ovaprene vaginal ring and one or two others, participants were advised to use condoms from the first day of the menses in the cycle in which the PCT would be performed. No intercourse or male ejaculation was allowed starting on about Day 10 of the cycle.
- Ovulation predictor kits:
  Home urinary test kits that assessed LH, and in some cases estradiol, were used in most studies to indicate the best time to find midcycle cervical mucus.
- Adequate mucus:
  The cervical mucus score, using the five WHO criteria, had to be at least 10 for the evaluation of sperm with this technique to be considered valid (Table 1). The absence of sperm in the mucus prior to the coital act being tested had to be documented in order to ensure that sperm seen after the coital act came only from that act. If no sperm were seen in the cervical mucus after coitus with the product, sperm had to be seen in the vagina to provide evidence that sex had actually taken place.
- Interval between coitus and mucus assessment:
  An interval of 2–3 h between the coital act and evaluation for sperm was usually used, based on Moghissi's assertion that "after ejaculation, sperm reach the level of the internal os rapidly. Their numbers increase gradually and reach a peak approximately 2 to 3 hours later." [10]. This interval is the most likely to detect any sperm that has made it through or around the chemical or mechanical barrier being tested. In addition, according to the WHO fifth edition manual, "Spermatozoa are usually killed in the vagina within 2 hours," thus this interval should be long enough to minimize the chance that motile vaginal sperm could contaminate the cervical sampling.
- Method of assessment of motile sperm:
  Sperm were counted in nine HPFs in a set pattern in an area representative of the distribution of sperm on the slide. In later studies, a gridded slide was used to facilitate sperm counting, and jelly containing microbeads was placed between the corners of the coverslip and the slide to standardize the height of the mucus sample.

Variations in PCTs for vaginal contraceptive testing

Likely due to the wide range of motile sperm associated with higher rates of pregnancy in infertility studies (from >1 to >20), these PCT studies varied in two important ways, both involving the average number of PMS/HPF.

First, in order to be eligible for the study, women had to have an average of ≥1, ≥5, or ≥10 PMS/HPF in the baseline cycle, depending on the study. Only the Advantage 24 study [31] required

Table 2. Vaginal chemical and mechanical barrier studies using PCTs carried out in tubally sterilized women

Test product and year of PCT study publication	Type of cycle	PCT studies: mean number of PMS/HPF. SD and range shown, if available	Contraceptive effectiveness study: 6-month typical use pregnancy rate, if available
Lea's Shield, 1995	Baseline [35]	>5[a] (n = 10)	
	Lea's Shield + N-9 [35]	0 (n = 10)	8.7% [40] (n = 146)
	Ortho diaphragm + N-9 [35]	0 (n = 10)	
Advantage 24 gel (N-9, 52.5 mg), 1996	Advantage 24, applied 15–30 min before coitus [22]	0.5 (n = 120) 2% of PCTs had ≥10 PMS/HPF	
	Advantage 24, applied 12 h before coitus [22]	2.5 (n = 111) 9% of PCTs had ≥10 PMS/HPF	
	Advantage 24, applied 24 h before coitus [22]	4.4 (n = 139) 14% of PCTs had ≥10 PMS/HPF	
	Conceptrol (N-9, 100 mg), applied 15–30 min before coitus [22]	0.1 (n = 127) All values < 10	
FemCap, 1997	Baseline [36]	Baseline cycle #1: 18.0 (n = 7) SD 20.5 Baseline cycle #2: 17.8 (n = 7) SD 17.8	
	FemCap + N-9 [36]	0.2 (n = 7) SD 0.4	13.5% [41] (n = 327)
	Ortho diaphragm + N-9 [36]	0 (n = 7)	7.9% [41] (n = 372)
BZK film, 1997	Baseline [31]	Baseline #1: 22.2 (n = 10) SD 20.2 Baseline #2: 21.2 (n = 10) SD 20.2	
	BZK film, 19 mg [31]	0.2 (n = 10) SD 0.6	
	BZK film, 25 mg [31]	0 (n = 10)	
	VCF film (N-9, 70 mg) [31]	0 (n = 10)	
N-9 film, 1997	Baseline [32]	Baseline #1: 23.7 (n = 10) SD 26.7 Baseline #2: 15.0 (n = 10) SD 14.6	
	N-9 film, 100 mg [32]	0.6 (n = 10) SD 0.9	
	N-9 film, 130 mg [32]	0.9 (n = 10) SD 2.3	
	VCF film (N-9, 70 mg) [32]	0.5 (n = 10) SD 0.8	
ACIDFORM gel (later Amphora and Phexxi), 2004	Baseline [34]	17.94 (n = 20) SD 19.91	
	ACIDFORM applied 0–30 min before coitus [34]	0.19 (n = 20) SD 0.52	13.7% [42] (n = 1183) (7-cycle cumulative typical-use pregnancy rate or Food and Drug Administration (FDA) reviewed. Amphora was applied 0–60 min before coitus.)
	ACIDFORM applied 8–10 h before coitus [34]	0.75 (n = 20) SD 1.37	
C31G gel, 2004	Baseline [33]	14.6 (n = 22) SD 9.0 Range: 5.0–36.3	
	C31G 0.5% [33]	0.3 (n = 13) SD 0.6 Range: 0–2.0	
	C31G 1.0% [33]	0.5 (n = 18) SD 2.0 Range: 0–8.3	12.0% [43] (n = 932)
	C31G 1.7% [33]	0.4 (n = 15) SD 1.6 Range: 0–6.1	
Ovaprene vaginal ring, 2009[2]	Ovaprene vaginal ring [37] (no baseline cycle conducted)	0 (n = 20)	
Caya (SILCS) diaphragm + N-9, 2008	Baseline [38]	12.5 Range: 5.9–35.6 (n = 14) SD 8.8	12.5% [44] (n = 128)
	Caya + N-9 [38]	0 (n = 8)	
Caya (SILCS) diaphragm + N-9, 2017	Baseline [39]	22.5 (n = 9) SD 33.4	
	Caya + N-9 [39]	0 (n = 9)	

[a]In baseline cycles, all participants were required to have at least five PMS/HPF to continue in the study. No further details about the average number of PMS/HPF in the baseline cycles were provided in the publication of this study.

BZK, benzalkonium chloride; HPF, high power field; PCT, postcoital test; PMS, progressively motile sperm; SD, standard deviation.

This table does not include the unpublished results of a new, recently completed PCT study on Ovaprene. According to a press release dated 11/12/19 from its new developer, Daré Bioscience, Inc., "The study enrolled 38 participants who completed a 'baseline PCT cycle' in which at least five PMS/HPF were observed in the woman's cervical mucus after intercourse with no contraceptive device in place... Twenty-three participants completed a total of approximately 21 visits each... The PCT clinical study met its primary endpoint - Ovaprene prevented the requisite number of sperm from reaching the cervix across all women and all cycles evaluated. Specifically, in 100% of women and cycles, an average of less than five (< 5) progressively motile sperm (PMS) per high power field (HPF) were present in the midcycle cervical mucus collected two to three hours after intercourse with Ovaprene in place." https://darebioscience.gcs-web.com/news-releases/news-release-details/dare-bioscience-announces-positivefindings-postcoital-test, accessed 1/26/20

≥10 PMS/HPF. The Lea's Shield study [36] required more than or equal to five PMS/HPF, the figure supported by Collins [21]. Subsequent studies (FemCap, BZK film, N-9 film, C31G, and Acidform) required only more than and equal to one PMS/HPF until the two Caya studies that returned to Collin's standard of more than and equal to five PMS/HPF (these are the two most recent studies performed as part of a registration package with the FDA).

Second, the primary endpoint, or definition of a satisfactory result in test cycles, meaning a decrease in PMS/HPF after product use, was variously set at <1, <5, or <10 PMS/HPF. The cut-off was <5 PMS/HPF in the Lea study and then <10 in the Advantage-24, FemCap, BZK film, and N-9 film studies, before being lowered to the cut-off of less than one PMS/HPF in the later Acidform study and less than five PMS/HPF in the C31G and two Caya studies.

A standardized method for PCT testing, including set baseline and post-product parameters, along with clinical trials determining actual pregnancy rates with product use will continue to improve

understanding of the correlation between PCT outcomes and product contraceptive effectiveness.

PCT prediction of pregnancy prevention

Not all products went to contraceptive effectiveness trials, but some did. The first three columns in Table 2 shows results of PCTs of vaginal contraceptives, including both the results of baseline cycles done without a product, and cycles in which a product was used. The pregnancy rates seen in contraceptive effectiveness trials, if carried out, are also shown in the fourth column.

It may be seen that the average number of PMS/HPF in baseline PCT cycles falls within the range of 12.5–23.7. With the exception of the low-dose N-9 gel Advantage-24 when it was applied 12–24 h before coitus, average values with a product in place are uniformly below 5 PMS/HPF—all are actually below 1.0 PMS/HPF and some are 0, although outliers with values of over 8 PMS/HPF exist. Standard deviations are somewhat wide due to the small number of subjects, but Glatstein found that among observers of identical slides, there was fair reproducibility (kappa statistic 0.40–0.75) for sperm number and motility [20]. Six-month typical use effectiveness rates vary, but all correspond to at least 86% effectiveness. It appears that a product that performs well in a PCT study goes on to demonstrate contraceptive effectiveness at a level *predictive* of a highly effective product, although not predictive of the exact effectiveness rate. For instance, Lea's Shield and the Ortho and Caya diaphragms, all had PCTs with an average of 0 PMS/HPF and typical use failure rates were 8.7, 7.9, and 12.5%, respectively. The ultimate contraceptive effectiveness is influenced by the ease and convenience of use of the product, along with patient compliance. Lea's Shield, FemCap, the Caya diaphragm, and Phexxi received FDA approval based on their contraceptive effectiveness studies.

Feasibility of PCT in clinical trials

Studies of products other than vaginal contraceptives often use correlates of protection as endpoints in Phase II studies. In evaluating vaginal contraceptives, the PCT is the closest thing we have to a correlate of protection—that is, something that gives an indication of whether the product works before it is tested in subjects at risk for the condition the product is supposed to prevent. However, PCT studies are extremely challenging in terms of scheduling—the woman and her partner must be able and willing to engage in intercourse on short notice and at a time that may not be at all conducive to it. In addition, the woman and the site staff must be available in the evening and on weekends for collecting the test samples, also on short notice. For these reasons, conducting a PCT study of the size expected for a typical larger Phase II study has not been deemed feasible in the development of any vaginal contraceptive to date.

Considerations for addressing current challenges

Because of these challenges, methods to facilitate PCT studies or use of surrogate markers are being considered. As previously mentioned, standardization of the PCT for baseline and post-use parameters will improve ability to predict pregnancy rates.

Other methods to facilitate the PCT could be beneficial. With respect to determining midcycle, levels of mucins in the cervical mucus, particularly Mucin 5b, and O-glycosylation of mucins have been shown to change during midcycle [45, 46]. The performance of mucins compared with ovulation predictor kits that assess both urinary LH and estradiol has not been studied, and due to the large increase in LH prior to ovulation, it is not likely that other methods would prove to be more accurate. However, for lab testing of cervical secretions, mucins could replace the WHO criteria for cervical mucus scoring if adequately studied.

Surrogate markers of the barrier properties of cervical mucus (e.g., pore size and microrheology) have also been studied. This would be helpful in the context of progestin-only contraceptive methods that thicken cervical mucus but do not consistently prevent ovulation. For products that make it more difficult for sperm to penetrate mucus, these markers, including particle tracking, could be useful preclinically to predict effectiveness of the method [47, 48]. In vitro testing could precede clinical studies by use of collected cervical secretions and sperm with particle tracking analysis to aid in product development. However, ultimately clinical trials will need to be performed, and the PCT would still be the best predictor.

Conclusion

Sangi-Haghpeykar wrote of the PCT that "this test is currently the best method available for estimating the performance of a spermicide in humans other than a full-fledged efficacy trial." [31]. It can be concluded that a PCT study of a test product, carried out in the same manner as recent PCTs before it, can be predictive of contraceptive efficacy. PCT results similar to results seen with products that later showed satisfactory performance in efficacy trials is currently the best indicator we have of likely success of the test product.

Conflict of interest

Christine Mauck is employed by Daré Bioscience, Inc., the developer of Ovaprene.

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