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Sperm-Cervical Mucus Interaction Test and Its Importance in the Management of Infertility

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ABSTRACT

Sperm-cervical mucus penetration test is a preliminary diagnostic test useful for infertile couples with no obvious anatomical and physiological abnormality. Cervical mucus constitutes first selection barrier for the selection of the healthiest sperm for fertilization of the ovum.

The sperm-cervical mucus interaction test is useful in accessing the incapability of the hostile cervical mucus. This test can also help physicians in diagnosis of antisperm antibodies in the partner. This chapter presents the importance of this simple but useful test in the diagnosis and management of infertility.

INTRODUCTION

Infertility, defined as the inability to achieve pregnancy after one year of unprotected intercourse, often is associated with abnormal semen parameters in the man or menstrual abnormalities in the woman. In some cases, however, infertility is evident despite a normal semen profile in the man and a normal menstrual cycle in the woman with no apparent physiological defect. The explanation in such cases may lie in abnormalities of the cervix. The cervix has been shown to play an important role in the sperm’s ability to permeate into the uterine cavity. Cervical abnormalities and hostility of the cervical mucus towards sperm are responsible for infertility in approximately 5-10% of women. The sperm-cervical mucus penetration test has emerged as a useful method for an initial screening of couples with immunological infertility and husbands who developed antisperm antibody against the cervical mucus of their wives.

After intercourse, semen is deposited into the vagina where seminal coagulum liquefies and the active sperms are released, passing through a complex biological fluid known as cervical mucus that is produced from
the secretory cells of the endocervix. The cervical mucus is considered to be the first selective barrier limiting the accessing active sperm to the female genital tract. As mucus covers the opening of the cervix, it prevents pathogens from ascending into the uterus. The fluid that is secreted into the vagina traps microorganism and flushes them out of the vagina, protecting both the uterine sterility and the vaginal epithelium.

Human cervical mucus consists of two parts; the aqueous part containing carbohydrates, amino acids, lipids, soluble macromolecules, locally produced proteins, peptides, polysaccharides, enzymes and hormones. The other component is a gel containing mucins, high molecular weight glycoprotein that forms a complex mesh-like structure. Throughout the menstrual cycle, the biophysical and biochemical characteristics of the mucus, as well as the quantity of the fluid, show significant changes. At midcycle, under the influence of estrogenic hormones, the amount of mucus increases, and it becomes more hydrated and less viscoelastic. These changes facilitate penetration by spermatozoa. When progesterone level increases, the mucus becomes less hydrated and more viscous and acts as a barrier to sperm. When mucus is most abundant, inorganic salts like NaCl along with K ions play an important role in forming organized crystallization, known as a ferning pattern.1,2 Some studies also indicated that abnormally thick cervical mucus that is resistant to sperm penetration is produced due to abnormal follicular growth, which is related to inadequate estrogen production.

Cervical mucus plays a major role in diagnosing sperm autoimmunity. Sperm cells with surface antibodies show impaired motility and agglutination, which hinders or prevents the penetration of spermatozoa through the cervical mucus. Several studies show a positive correlation between sperm autoimmunization and a poor result of the postcoital in vivo sperm-cervical mucus penetration test. On the other hand, three types of immunoglobulins (IgA, IgG, IgM) are present in cervical mucus that act as antibodies and may cause immunological reactions with sperm surface antigens. This might result in death or agglutination of sperm, leading to infertility. The cervical mucus penetration test enables the physician to identify the incompatibilities of hostile mucus and antisperm antibodies.

The use of human mucus for in vitro penetration tests presents several difficulties, including the problem of collecting relatively large quantities of the fluid from the endocervix and wide variation in viscosity.2,5 Several substitutes for mucus are in use, including bovine cervical mucus, which has similar biophysical and biochemical properties and is available in large amounts and hyaluronic acid, which is a mucopolysaccharide that is structurally similar to human cervical mucus.6 Other substitutes also have been proposed.
COLLECTION AND PRESERVATION OF CERVICAL MUCUS

Collection of cervical mucus from the endocervix is performed without anesthesia under aseptic conditions. All equipment including the speculum, forceps, cotton, etc. should be sterilized properly. The uterine cervix is exposed by sterilized speculum, and the exocervical region is thoroughly cleaned by sterile cotton swab to remove the exocervical mucus and vaginal contaminants. This should be done by a clinician or certified trained nurse. Mucus is obtained from the endocervix by gentle aspiration with the help of a plastic sterilized Pasteur pipette, sterile tuberculin syringe without needle or polyethylene tube, etc. During collection, suction should be maintained without causing trauma to the uterus. Bubble formation and vaginal interference should be avoided to ensure an accurate evaluation.

After collection, the properties of the mucus should be tested on the day of collection. If this is not practical, the sample must be preserved properly until it is tested. Mucus can be preserved directly in the aspiration tool or in a small microfuge tube that is sealed by plasticine to avoid dehydration, contamination with air or microorganisms if the storage container is stored at 4ºC up to 5 days before analysis.

CERVICAL MUCUS EVALUATION

The fertile period can be predicted by the mucus quality across the ovulatory period. At an appropriate time during the menstrual cycle, sperm cells can migrate through the cervical mucus. This period of time varies among women and in the same individual from one cycle to another. Cervical mucus is an egg white-like viscous fluid, which increases in amount and normally takes a watery like appearance around the time of ovulation.

Cervical mucus evaluation includes assessment of volume, spinnbarkeit, ferning [crystallization], consistency, cellularity and pH. A scoring procedure for cervical mucus was postulated by Moghissi in 1976, based on the original proposal of Insler et al. The pH of the cervical mucus is excluded from the scoring criterion. The final score is obtained by adding the individual scores of each category. A score greater than 10+ is considered good cervical mucus; a score less than 10+ is considered unfavorable cervical mucus. Maximum score is 15+.

Scoring Parameters

1. Volume

The volume is scored as follows:

- 0 = 0 ml
- 1+ = 0.1 ml
- 2+ = 0.2 ml
- 3+ = or > 0.3 ml
2. **Consistency**

Cervical mucus viscosity is measured by scoring consistency. Viscosity decreases during ovulation, which facilitates sperm penetrability. Higher viscosity cervical mucus that is more resistant to sperm migration is observed in the luteal phase of the menstrual cycle.

Consistency is scored as follows:

- 0 = thick, highly viscous, premenstrual mucus
- 1+ = mucus of intermediate viscosity
- 2+ = mildly viscous mucus
- 3+ = watery, minimally viscous, midcycle (preovulatory) mucus

3. **Ferning**

The crystallization pattern of cervical mucus is observed by spreading the mucus on a glass slide that is air-dried and observed under 400× magnification. This crystallization pattern, or ferning, is due to the presence of inorganic salts such as NaCl and K ions. Fern structure also varies with the composition of the cervical mucus. A primary stem and secondary, tertiary and quaternary branching are observed under the influence of hormones and ions of the cervical mucus.

Ferning is scored as follows:

- 0 = absent
- 1+ = initiation of fern formation
- 2+ = primary and secondary stems
- 3+ = tertiary and quaternary stems

(Figure 12.1)

4. **Spinnbarkeit**

Spinnbarkeit is evaluated by measuring the distance in centimeter that mucus can be stretched with the aid of forceps.

Spinnbarkeit is scored as follows:

- 0 = 1 cm
- 1+ = 1-4 cm
- 2+ = 5-8 cm
- 3+ = 9 cm or more

(Figure 12.2)

5. **Cellularity**

Leucocytes and other cells present in the mucus are evaluated in terms of 0 to 3+.

- 3+ = no cells are observed in each high power field
Figure 12.1: Human cervical mucus ferning with tertiary and quaternary stems

Figure 12.2: Spinnbarkeit test

2+ = 1-10 cells/HPF
1+ = 11-20 cells/HPF
0 = more than 20 cells/HPF

The final score is obtained by adding the scores of the individual parameters.
SPERM-CERVICAL MUCUS INTERACTION TEST

pH

Cervical mucus pH is determined by placing a drop on pH paper. The normal pH range of midcycle cervical mucus is 7-8.5. Proper assessment of cervical mucus pH is necessary, as it significantly affects sperm motility and viability. Slightly alkaline mucus facilitates sperm motility; acidic pH may indicate abnormal secretion of cervical mucus or bacterial infection. The possibility of contamination through vaginal secretion due to improper and careless collection methods should always be ruled out.

Post-coital Test (PCT)

This is an in vivo cervical mucus interaction test. The most appropriate time for the test is 2-5 hrs after intercourse, since the largest sperm population in the mucus is normally found at this time. The post-coital test should be performed prior to ovulation, with the optimal timing just before ovulation. Ovulation can be predicted by clinical investigation, such as basal body temperature, cervical mucus changes, and ultrasound results for follicular size determination or hormonal status. The purpose of the study is to evaluate sperm survivability and activity, as well as cervical mucus hostility.

Procedure for PCT

Patients are instructed to abstain from intercourse for at least 2 days before the day of the test, and to avoid the use of lubricants during or after intercourse or soap for bathing. A nonlubricated, sterilized speculum is inserted to expose the vagina. The vaginal sample should be cleared thoroughly by a tuberculin syringe/plastic Pasteur pipette or a polyethylene tube, which should be examined to ensure semen deposition. After the mucus sample is aspirated from the endocervical canal, it is placed onto a clean slide and covered with a cover slip, avoiding bubble formation. The sample is examined under light microscope at 100 × followed by examination at 400 ×. The number of spermatozoa in the mucus is counted by taking 20 fields, and a mean is taken.

Evaluation of Sperm Concentration and Motility

PCT is considered “good” when at least two motile spermatozoa of high progressive motility are observed in preovulatory cervical mucus, 8-12 hrs after intercourse; otherwise, it is classified as poor. For further detailed evaluation and reporting, PCT results are subdivided in four groups:

Negative: No spermatozoa found in mucus/LPF, but present in vaginal secretion.
Inadequate: Two motile sperm/HPF

Moderate: Two to six motile sperm/HPF

Excellent: Seven or more motile sperm/HPF.

Motility of spermatozoa is graded as follows:

- a = rapid progressive motility
- b = slow or sluggish progressive motility
- c = nonprogressive motility
- d = immotile spermatozoa

The presence of any rapidly progressive motile spermatozoa in the cervical mucus is an indicator of normal spermatozoa. Negative or abnormal PCT results may be observed due to improper deposition of semen into the vagina or incorrect timing. In the event of a negative or abnormal result, the PCT should be repeated several times during the same cycle.

**In Vitro Cervical Mucus Penetration Tests**

Negative or abnormal PCT results are an indication for performing *in vitro* cervical mucus penetration tests. Two different techniques have been used for *in vitro* investigation of sperm penetration: (1) The simplified slide method (2) the capillary tube technique. The *in vitro* sperm mucus penetration test (SMPT) is done to measure the ability of the spermatozoa to migrate into a column of mucus or substitute.8

**In vitro Test**

The *in vitro* test is performed after repeated abnormal post-coital test results. This test is more useful when donor semen and donor cervical mucus are used to verify the source of the abnormal/incorrect results obtained using both the husband’s sperm and the wife’s cervical mucus.

**Sample Collection**

A semen sample is collected from the husband or a male donor after abstinence from intercourse at least for 2 days. Cervical mucus is collected from the wife or a female donor who is undergoing artificial insemination in her natural cycle or is being treated by gonadotrophin. Patients taking clomiphine citrate are excluded from the study because of its possible effect as an antiestrogen on the cervix. The test ideally should be done within 30 to 60 minutes after semen collection. Collection of a midcycle human cervical mucus under sterile condition is essential for proper assessment.
**Simplified Slide Test**

A moist chamber is prepared for placement of the glass slide. A drop of cervical mucus is placed on a clean glass slide and covered with a cover slip (22 mm × 22 mm), avoiding bubble formation. A drop of semen is deposited at the each side and in contact with the cover slip so that semen can move into the mucus by capillary action. The slide is placed in the moist chamber and kept at 37°C for 30 min. After 30 minutes, it is observed under bright field microscope where a finger-like projection is observed at the semen-mucus interface. Spermatozoa enter into the cervical mucus after crossing the interface. This is a qualitative method, and this test cannot be used to quantify the number of cells present in the mucus or to measure the size and shape of the interface.

It is scored as follows:

- **Normal:** > 90% cells are motile with rapid forward movement.
- **Poor:** Slow progressive motile cells.
- **Abnormal:** Most of the sperm cells are immotile, lack forward progression or exhibit shaking movement, which may indicate the presence of antisperm antibodies.
- **Zero:** No penetration is observed.

**Capillary Tube Test**

This test measures the ability of sperm in the semen to swim up into a column of cervical mucus.

**Method**

A flat capillary tube 5 cm × 3 mm × 0.3 mm is normally used for this test. Midcycle human cervical mucus is aspirated directly into the capillary tube, avoiding bubble formation. One end of the tube is sealed with plasticine, and the open end of the tube is placed in a 1.5 ml microfuge tube (Appleton Woods, Birmingham, UK) containing 100 µl of the liquefied semen sample. The semen reservoir and the capillary tube are placed on a microscope slide in horizontal position as described in the WHO manual (1999). After incubation for 1 hour at room temperature while maintaining the humidity, the capillary tube is observed under bright field illumination with 200 × magnification, Scanning of the capillary tube under the microscope establishes the distance furthest from the semen reservoir attained by the spermatozoa. The migration distance is defined as the maximum distance in centimeters covered by spermatozoa after 1 hour of incubation. Sperm penetration concentration is assessed at half the migration distance using the same magnification and counting spermatozoa while focusing from
the lower to upper wall of the capillary in a single pass. The penetration score is calculated by multiplying half the migration distance by the number of spermatozoa counted at half the migration distance. Sperm motility is assessed by examining at least 200 spermatozoa at half the migration distance.\textsuperscript{10,11}

REFERENCES