REVIEW

Insights into the role of cervical mucus and vaginal pH in unexplained infertility

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Unexplained infertility diagnosis is made in the presence of a normal semen analysis when tubal patency and normal ovulatory function are established. Among several potential causes, unexplained infertility could be attributed to vaginal pH and cervical mucus abnormalities. Although the vaginal canal and the cervix generally function as effective barriers to sperm, and although the production of mucus is essential to transport them from the vagina to the uterine cavity, these factors receive little attention in the investigation of couples with unexplained infertility. A substantial reduction in sperm number occurs as they transverse the cervix. From an average of 200 to 300 million sperm deposited in the vagina, only a few hundred achieve proximity to the oocyte. Given this expected high spermatozoa loss, a slight modification in cervical mucus may rapidly transform the cervix into a "hostile" environment, which, together with changes in vaginal environment and cervix structure, may prevent natural conception and be a cause of infertility. In this review, we discuss the physiological role of the vaginal pH and cervical mucus in fertility, and describe several conditions that can render the cervical mucus hostile to sperm and therefore be implicated in the pathophysiology of unexplained infertility.

KEYWORDS: Sperm transport; Cervix mucus; Hydrogen ion concentration; Sperm agglutination; Uterine cervix disease; Vaginal disease; Female infertility; Unexplained infertility.

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INTRODUCTION

Infertility is customarily defined as failure of a couple to conceive after 12 months of regular unprotected intercourse. After extensive evaluation of both partners, 20–30% of infertile couples remain childless without identifiable causes, according to the routinely used tests, and are classified as having unexplained infertility. Potential etiologies of unexplained infertility encompass miscomprehensions on the part of the couple regarding the concept of the female fertile window, improper coital techniques, erectile dysfunction, as well as molecular and functional causes of male and female infertility. Although it is well established that the vaginal pH and cervical mucus play important roles in maintaining sperm function after intercourse, their importance in unexplained infertility is generally underestimated.

The optimal vaginal pH to maintain sperm viability and motility ranges from 7.0 to 8.5.[1,2] In fact, a reduction in sperm motility is seen at vaginal pH of less than 6.0.[2–5] The alkaline pH of semen protects sperm temporarily. However, reduction in semen volume and/or decreased alkaline seminal vesicular secretion may negatively impact fertility since buffering capacity of semen against vaginal acidity becomes inadequate.[6]

The cervix generally function as an effective barrier to sperm; an adequate production of cervical mucus is essential to transport sperm from the vagina to the uterine cavity.[2,7] Surgeries, birth defects and infections can cause cervical constriction or stenosis, and may impair mucus production by the endocervical canal.[8–12] Chronic cervicitis, acute inflammation and congenital diseases, such as cystic fibrosis, are among the conditions that can reduce cervical mucus receptivity.[12–14] As a result, fertility is impaired due to alterations in cervical anatomy and function.[9] Hormonal fluctuations during the menstrual cycle also impact the production, composition and ultrastructure of mucus, which ultimately affect sperm penetrability.[12,15] Hormonal dysfunctions, mainly characterized by inadequate estrogen production and/or premature progesterone elevation, may render cervical mucus inadequate for sperm penetration, and may result in infertility.[9] Medication (e.g. clomiphene citrate, propranolol) and smoking can also have detrimental effects on mucus receptivity to sperm.[16–20] Lastly, sperm abnormalities affecting motility and/or morphology, as well as elevated levels of antisperm antibodies in the semen and/or in the female serum may impact ability of sperm to transpose the cervical mucus.[21–23]

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Altogether, the aforementioned factors highlight the importance of both vaginal pH and cervical mucus as the first barrier to sperm penetration into the uterine cavity. In this review, we first describe vaginal physiology. Then, we characterize the cervical mucus, including its production, structure and composition. Lastly, we explain how spermatozoa are transported into the cervical mucus and outline several conditions that can interfere with sperm movement through the vagina and cervical mucus and, therefore, be implicated in the pathophysiology of unexplained infertility.

VAGINAL PHYSIOLOGY

Vaginal pH

Potential of hydrogen, or pH, is the standard measure of hydrogen ion concentration, the quantitative appraisal of the acidity or alkalinity of a solution. Numerically, it is equal to 7.0 for neutral solutions. Levels of pH less than 7.0 characterize acidic solutions, while levels greater than 7.0 characterize basic (or alkaline) solutions. The vaginal pH fluctuates from 3.8 to 4.5, and is classified as moderately acidic.[1]

The fluid content of the vagina is derived from:

1. Mucus secretions of the cervical columnar cell;
2. Transudation through the vaginal walls;
3. Vulvar secretions originated from sebaceous and sweat glands;
4. Mucus secretion of Bartholin's glands;
5. Substances produced by microorganisms present in the vagina.[24–26]

The vagina is a genital canal that extends from the vulva to the cervix. Its walls consist of non cornified stratified squamous epithelium, a smooth muscle layer and a prominent connective tissue layer, rich in elastic fibers.[27] The epithelial cells are rich in glycogen. Vaginal cells are stimulated by estrogen to both synthetize and accumulate increased amounts of glycogen. Due to cell shedding and desquamation, glycogen accumulates in the vaginal lumen. Glycogen can be metabolized in a process called glycogenolysis to pyruvic acid, which is converted to lactic acid and water by anaerobic metabolism. This process is carried out by Doderlein's lactobacillus, the predominant vaginal microorganism, thus decreasing the vaginal pH. As such, the combination of epithelial cells rich in glycogen and the presence of the lactobacillus are essential to maintain vaginal acidity.[24,25] This acid environment protects the vagina from pathogenic microorganisms because most bacteria grow best at a pH of about 7.5.[24]

Lactobacilli also protect the vagina by competing with other bacteria for adherence to the vaginal epithelium, thus forming a biofilm on the cervical and vaginal mucosae. Furthermore, lactobacilli produce antimicrobial substances such as hydrogen peroxide, bacteriocins and biosurfactants.[26] Many factors can interfere with the number of Doderlein's bacilli, and consequently, modulate vaginal pH, such as the systemic or topic use of antibiotics, stress, immunity decrease, hormonal disorders and modifications in estrogen levels during a woman's lifetime.[28]

As a result of fetal exposure to maternal-placental estrogens in the first month of life, lactobacilli are abundant in the vagina, thus maintaining vaginal pH around 5. From the first month of life until puberty, the glycogen content of the vaginal epithelial cells decreases in response to decreased estrogen levels. Consequently, the production of lactic acid decreases while vaginal pH rises to about 7. This modification facilitates the growth of other bacteria, mainly *Staphylococcus epidermidis*, *Streptococcus* and *E.coli*.[28] Estrogen levels increase again during reproductive years, due to the onset of ovarian activity, lowering vaginal pH to less than 5. This decrease in vaginal pH predisposes to the proliferation of lactobacilli, which accounts for 90% of all microorganisms present in the vagina at this time. Other bacteria such as *Corynebacterium*, *Staphylococcus*, *Streptococcus*, and *Bacterioides* make up the remaining 10% of this flora.[29]

During the menstrual cycle, the vaginal pH becomes more acidic from the 2nd to the 14th day of the cycle, ranging from 6.6 ± 0.3 to 4.2 ± 0.2.[29] This acidic vaginal environment is toxic to sperm, because the optimal pH for sperm viability ranges from 7.0 to 8.5, and a reduction in sperm motility is seen at pH of less than 6.0.[2–5] During sexual intercourse and as a result of sexual excitement, the vaginal epithelium produces a transudate that lubricates and also elevates the vaginal pH to 7.0 within seconds. This decrease in acidity can be maintained for up to two hours after ejaculation.[6] This physiological modification, associated with the alkaline pH of semen, temporarily protects spermatozoa.[30]

The vaginal pH also increases during menses, because blood is slightly alkaline, and also in patients with excessive cervical ectropion, which produces alkaline mucus.[31] Postmenopausal women have a lower amount of glycogen in the epithelial cells as a consequence of hypoestrogenism, and their vaginal pH is around 7, a condition similar to what is found in prepubescents. Increased vaginal pH in the aforesaid conditions predisposes to proliferation of pathogenic bacteria.[32]

Variations in the vaginal pH diminish its defense and increase its susceptibility to infections, which can indirectly affect fertility. An association of abnormal vaginal flora (bacterial vaginosis) with increased tumor necrosis factor alpha (TNF-α) and interferon-gamma (IFN-γ) levels in the cervical mucus has been described in patients with unexplained infertility.[33] In addition, concentrations of TNF-α and IFN-γ are significantly higher in the cervical mucus of infertile women with unexplained infertility compared with fertile controls.[34] These data suggest that an altered vaginal ecosystem can induce the production of proinflammatory cytokines which may play a role in the pathophysiology of unexplained infertility.[33] The mechanism by which these cytokines impair fertility is still unclear; however, it has been found that high levels of TNF-α and IFN-γ are associated with elevated levels of activated natural-killer cells.[35]

Lastly, semen disability to neutralize the acidic vaginal pH can also be an infertility factor because spermatozoa are vulnerable to vaginal acidity.[6] For instance, an abnormally low semen volume (hypospermia) negatively impacts fertility since the semen buffering capacity against vaginal acidity is decreased. The same is true when semen becomes acidic, which may occur as a result of obstruction in the ejaculatory ducts and due to hypoplasic seminal vesicles.[36]

Sperm transport at the vaginal level

Under normal conditions, only about 200 out of approximately 280 million spermatozoa deposited in the upper vagina upon ejaculation are capable of successfully traversing the cervical canal.[37] Almost immediately after ejaculation, the semen forms a coagulum that temporarily restricts the movement of sperm out of the seminal clot. Then, this coagulum is gradually liquefied during the next

20–30 minutes by seminal-fluid proteolytic enzymes produced by the prostate gland.[2,38,39] Deficiency in prostatic secretions, usually caused by infectious processes, is related with the absence of secondary liquefaction or partial liquefaction. As a consequence, clustered sperm are trapped within highly viscous semen, which can impair the sperm ability to transverse the cervix.[9,40]

Most spermatozoa are lost at the vaginal level with the expulsion of semen from the introitus. However, a variable number of spermatozoa are rapidly taken up by the cervical mucus in a process described as "rapid transport", leaving behind the seminal plasma.[2,7] Rapid sperm transport may begin within seconds after ejaculation. In fact, spermatozoa are found in the mucus within 90 seconds post-ejaculation.[38] Despite helping sperm reach the cervical mucus, sperm motility is not the main drive for sperm transit. Sperm movement is predominantly passive, resulting from coordinated vaginal, cervical, and uterine contractions that occur during coitus. Although these contractions are of short duration, they are believed to be the primary force responsible for the rapid progression of sperm to the upper female reproductive tract, as occurs in other mammalian species.[2,41] Although there are reports of motile sperm persisting within the vagina for up to 12 hours after ejaculation, motility of most vaginal sperm is diminished within about 30 minutes, and after 2 hours almost all sperm motility has been lost.[39]

Other non-physiological factors may play a role in sperm loss at the vaginal level. The use of vaginal lubricants during coitus, for instance, has been shown to be toxic to sperm.[42–45] Vaginal infectious processes increase the number of leukocytes in the vagina, thus enhancing sperm phagocytosis and reducing the number of spermatozoa that enter the tongues of cervical mucus extended over the ectocervix.[7]

CERVIX AND CERVICAL MUCUS

Cervix

The cervix, which is the lower narrow portion of the uterus where it joins with the top end of the vagina, generally functions as an effective barrier against sperm.[7] However, several important functions have been attributed to the cervix and its secretion, including:

1. Protecting sperm from the hostile environment of the vagina;[31,46,47]
2. Protecting sperm from phagocytosis by vaginal leukocytes;[2,7,46,47]
3. Preventing sperm, microorganisms and particulate matter to access the upper reproductive tract and thus, the peritoneal cavity;[2]
4. Facilitating sperm transport during the periovulatory period and modulating at other cycle periods;[2,7,46,47]
5. Filtrating morphologically normal sperm;[2,7,46,47]
6. Preserving large numbers of sperm within the cervical crypts, providing a biochemical environment sufficient for sperm storage, capacitation, migration, and release of sperm into the upper genital tract.[2,7]

The anatomical and functional structure of the human cervix facilitates the performance of these aforesaid functions, but the production of mucus is probably the most important one. Throughout the menstrual cycle, the cervix changes in size and texture. Just prior to ovulation and as a result of the rise in estrogen levels, the cervix swells and softens, while its external os dilates. Also, during this time, the cervix secretes more abundant, slippery, clear and stretchy mucus, which exudes from cervix into the vagina, thus facilitating the entrance of sperm into the uterine cavity.[2,48] In the periovulatory period more than 96% of the cervical mucus is water, thus conferring the mucus high spinnbarkeit and pronounced ferning capacity; as such, sperm penetrability is highest at this time.[46] After ovulation, progesterone induces the cervix to harden, close and secrete thicker mucus, which acts as a plug, preventing bacteria and sperm from entering the uterus and making fertilization very unlikely.[48]

The endocervical canal is lined by single layer of columnar epithelial cells, both ciliated and nonciliated. The cervix does not contain true glandular units; instead, the epithelium is thrown into longitudinal folds and invaginations with blind-ending tubules arising from the clefts forming crypts off the central canal. The nonciliated cells secrete mucin in granular form through exocytosis. There are several hundred mucus-secreting units in the cervical canal. The daily production varies in relation to the cyclical changes of the menstrual cycle, from 600 mg during midcycle to 20–60 mg during other periods of the cycle. A few ciliated cells among the secreting cells propel the cervical mucus from the crypt of origin toward the canal.[2,12,49]

An uncommon cause of cervical infertility is a previous surgery on the cervix such as cryo- or electric cauterization, cone biopsy and loop electrosurgical excision procedure. These interventions can alter the anatomy of the cervix canal and may lead to constriction or even stenosis. As a result, the production of mucus may be impaired due to the removal of secretory cells.[8,9] Severe infections can also damage the mucus producing cells.[12] Interventions, such as curettage, can also block the canal or simply turn it into a pinpoint opening.[9] Birth defects can likewise affect the cervix. Most of such defects occur in women whose mothers had used diethylstilbestrol, a synthetic nonsteroidal estrogen, which was banned from the marketplace in 1997.[11] Anomalies in the Müllerian ducts, which differentiate to form the fallopian tubes, uterus, the uterine cervix, and the superior aspect of the vagina, can also result in a defective cervix. Most Müllerian duct anomalies are associated with functioning ovaries and age-appropriate external genitalia. These abnormalities are often recognized after the onset of puberty, but late presentations may include infertility.[10,50] All of these conditions may significantly impair the ability of sperm to trespass the cervix and enter the upper female reproductive tract.[8–12,50]

Cervical mucus

Cervical mucus is a heterogeneous mixture of secretions whose rate of production depends on several factors. These factors include the number of mucus-secretory units in the cervical canal, the percentage of mucus-secreting cells per unit and the secretory activity of the cells in response to circulating hormones.[12]

There are several types of mucus, as characterized by Odeblad.[51] Type E is thin and watery (with approximately 98% of water), which is characteristic of estrogen dominance. Type G is thick and sticky, and reflects the stimulation of progestogenic hormones. Under the influence of progesterone, the water content decreases to approximately 90% and the mucus becomes more viscous. Therefore, type E is predominant at the time of ovulation in a proportion of

about 97% of type E and 3% of type G, while type G predominates during the normal luteal phase.[12,15,51] Both types are always present in different proportions during the menstrual cycle, varying according to the levels of circulating progesterone and estrogen. Using nuclear magnetic resonance analysis, Odeblad and others established that the ovulatory mucus (E) is a mosaic composed of mucus "strings" (called Es) and "loaves" (labeled as El). The strings (Es) are fluid gels, and the loaves (El) are more viscid. The Es–El system is very dynamic. Ovulatory mucus contains 20–25% type Es, 72–77% type El and 3% type G. Since Es and El differ in their molecular architecture and their protein content and not all areas of the cervical mucus are equally penetrable by the sperm. While the Es mucus conveys the spermatozoa from the vaginal pool, the El type has a very limited role in this respect.[12,52,53] The differences between each type of mucus can be observed in dried mucus samples studied under light microscopy. Cervical mucus forms fern-like patterns due to the crystallization of sodium chloride on its fibers, which varies according with the mucus type.[54]

Ultrastructurally, cervical mucus can be seen as a complex biphasic fluid with high and low viscosity components. It is a hydrogel composed of a low-molecular-weight component (cervical plasma) and a high-molecular-weight component (gel phase). The cervical plasma consists mainly of trace elements (zinc, copper, iron, manganese, selenium, sodium and chloride ions), organic components of low molecular weight such as glucose and amino acids, and soluble proteins, such as albumin and globulins.[12,55,56] The gel phase consists of a glycoprotein network called mucin, presenting glycosylation variations according to the menstrual cycle, which contribute to the changes in its physical properties. This extremely large macromolecule (about 10,000 KDa) is rich in carbohydrate content and is responsible for the high viscosity of the mucus.[57] The mucin macromolecules are thread-like and appear in long parallel bundles held together by a peptide of 30 kDa. This peptide connects the mucin molecules through disulphide bridges (S–S), thus forming mucin micelles of 100 to 1000 glycoprotein chains.[55] This system assembly varies both in diameter and arrangement. Collectively, mucin molecules form a complex of interconnected micelles, which comprise a lattice whose interstices are capable of supporting the low viscosity phase, which is predominantly water. The protein content is low in the intermicellar spaces of Es mucus. The very low viscosity of Es intermicellar fluid allows very rapid sperm migration.[12,58] In type G mucus, no micelle formation occurs, but the long macromolecules form a large, three-dimensional, irregular, dense network that does not allow spermatozoa to penetrate.[59] These channels or spaces vary in size according to the type of mucus: 2–5 μm wide (Es type mucus); 1–2 μm wide (El type mucus) and 0.3–1 μm wide (type G mucus). Therefore, intermicellar spaces play a key role in sperm migration.[12]

Abnormalities of cervical mucus can result in infertility. For instance, chronic cervicitis is associated with alterations of cervical mucus. In this case, a different mucus pattern appears, defined as type Q by Odeblad,[51,52] in which the mucus composition varies depending on the type, degree and duration of the inflammatory process. The crypts releasing this type of secretion have limited response to hormonal stimulation.[51,60] In acute inflammatory conditions the crypts can also produce a serous type of secretion of low viscosity but with high leukocyte content, classified as type V, which is unable to maintain sperm vitality. Therefore, common infections of the cervix such as those caused by sexually transmitted microorganisms (*Chlamydia trachomatis, Neisseria Gonorrhea, Trichomonas vaginalis, Mycoplasma hominis* and *Ureaplasma urealyticum*) may result in cervical hostility.[12]

Women with cystic fibrosis are also unable to produce the watery and stretchy mucus needed for optimal sperm penetrability. Cystic Fibrosis is caused by a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator. This protein functions as a channel, which transports negatively charged particles (chloride ions) inside and outside the cells. The transport of chloride ions helps control the movement of water in tissues, which is necessary for the production of thin, freely flowing mucus. When this transmembrane conductance regulator protein does not work, chloride (Cl^-) is trapped inside the cells. Because chloride is negatively charged, it creates a difference in the electrical potential inside/outside the cell causing sodium to cross into the cell. As a result, water movement from inside to outside cellular compartments is decreased, leading a more viscous and less watery mucus which harms sperm transport. Along with a loss of Cl^- conductance, mutations of transmembrane conductance regulator protein also impede bicarbonate (HCO_3^-) transport. A HCO_3^- rich alkaline pH environment is crucial for optimal sperm motility and capacitation. During the process of releasing highly condensed mucins from intracellular granules, calcium (Ca^{++}) and hydrogen (H^+) cations must be removed to enable the mucins to expand by as much as 1000 times, forming extracellular mucus-gel networks. It is suggested that HCO_3^- is essential to normal mucin expansion because it forms complexes with these cations. Due to defective HCO_3^- secretion in cystic fibrosis, mucins tend to remain aggregated, poorly solubilized, and less transportable. It is tempting to consider that some cases of reduced fertility in females might be associated with putative mild mutations in this gene with consequent abnormal cervix-uterine mucus release due to inadequate HCO_3^- secretion.[14,61,62] An example of this condition is the report of two infertile sisters with significantly abnormal cervical mucus who were found to be compound heterozygote carriers of the cystic fibrosis ΔF508 and R117H/7T mutations.[13]

Many exogenous factors can render the cervical mucus hostile to sperm and therefore be implicated in the pathophysiology of unexplained infertility.[12] Clomiphene citrate, frequently used to stimulate follicle growth and ovulation as a first line therapy in couples with unexplained infertility, can interfere with the cervical mucus. Clomiphene citrate is structurally similar to estrogen, which allows it to bind to estrogen receptors throughout the reproductive system. In contrast to estrogen, clomiphene citrate binds to nuclear estrogen receptors for extended periods of time, that is, weeks rather than hours, which ultimately depletes these receptors by interfering with the normal process of replenishment. Acting at the hypothalamic level, clomiphene citrate is effective in ovulation induction by inhibiting negative feedback of estrogen on gonadotropin release, leading to up-regulation of the hypothalamic–pituitary–adrenal axis; this, in turn, serves to drive ovarian follicular activity. At the same time, clomiphene citrate exerts undesirable and unavoidable adverse antiestrogenic effects

in the periphery (endocervix and endometrium). Several studies have reported that clomiphene citrate has adverse effects on the quality and quantity of cervical mucus based on cervical mucus score, the value of which is debatable. Despite that, available evidence and accumulated clinical experience support the notion that any adverse antiestrogenic effect presents a significant obstacle for a very large majority of women treated with ovulation induction drugs.[16–18,63,64] Another drug that deserves attention is propranolol, which accumulates extensively in the cervical mucus after oral administration; its concentration is fourfold higher in the mucus compared with that in blood. Despite not affecting mucus production, propranolol accumulation may impair sperm motility by its direct effect on sperm membrane ion transport and energy production.[19,65–67] Nicotine, and its metabolite cotinine, are secreted into the cervical mucus, and can be found in the mucus even of passive smokers.[68,69] A retrospective study evaluating smoking histories of 901 women with infertility due to different etiologies and 1,264 pregnant women admitted for delivery suggested that smoking is a risk factor for cervical factor infertility (relative risk = 1.7; 95% confidence interval of 1.0 to 2.7); however, its mechanism of action is unclear.[70] It has been suggested that nicotine could have toxic effects on spermatozoa, but *in vitro* studies have noted that the harmful effects of nicotine and cotinine to sperm occurs in extremely high concentrations, not seen in the seminal plasma or cervical mucus of smokers.[20,71]

Other components of cervical mucus

Cervical mucus contains not only mucin but also other proteins such as albumin and globulin. The concentration of different proteins in mucus varies during the menstrual cycle, being lowest at ovulation. Morales et al, studying the mucus' concentration of protein and its ability to sustain sperm migration, found that peri-ovulatory mucus exhibits low protein concentrations as revealed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Most of the soluble proteins found in the cervical mucus had their lowest concentration around the peri-ovulatory period, when the mucus is most receptive to sperm penetration.[72] The authors of the aforementioned study concluded that there is a statistically significant inverse relationship between protein concentration in the mucus and its ability to sustain sperm migration.

Mucus also provides local immunity through a unique interaction of immunoglobulins (mainly IgA), cytokines and reproductive hormones (estrogen and progesterone). Also, mucus is a rich source of antimicrobial proteins and peptides, including secretory leucocyte protease inhibitor (SLPI), lysozyme, calprotectin, lactoferrin, human neutrophil peptides 1 to 3, and epithelial beta-defensin.[12,73] Increased levels of cervical mucus IgA and IgG have been reported in 23 women with genital infections caused by *N. gonorrhea*, *T. vaginalis*, genital herpes and nonspecific cervicitis in comparison with a control group of 23 uninfected women ($p < 0.001$), thus indicating increased local immune response.[74] Furthermore, it has been suggested that leukocytes, mainly neutrophils, play a role in both the cervical cellular defense line and the 'selective' mechanisms of sperm transport through the cervix (phagocytosis of abnormal spermatozoa).[75]

Prostaglandins and trace elements also have hormone-dependent cyclical variation in the cervical mucus during the menstrual cycle. Prostaglandins found in the cervical mucus are PGE_1, PGE_2, PGD_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$, and their contents increase in the pre-ovulatory period. However, their biological importance remains unclear.[76] Ryantová et al, evaluating PGE_2 levels in ovulatory cervical mucus of 120 women with unexplained miscarriages, found that PGE_2 levels were 6x, 13x and 21x higher in patients with one, two and three or more miscarriages compared with controls ($P < 0.033$), respectively.[77] Iron and copper levels show marked fall from pre-ovulatory to ovulatory phases. Interestingly, their levels in the cervical mucus were elevated both in patients with primary or secondary infertility couples compared with fertile counterparts. The real influence of these elements in fertility remains unclear, but a spermatotoxic effect of copper has been described.[55,78–80]

Table 1 summarizes the conditions that may affect fertility at vaginal and cervical levels.

SPERM TRANSPORT THROUGH THE CERVICAL MUCUS

Sperm movement inside the cervical mucus occurs primarily through the interstitial spaces in the mucin micelles. Sperm progression depends mainly on the size of these spaces.[81] The spaces between these large glycoproteins reach their maximum at the mid-cycle estrogen peak, when there is an increase in mucus production and in its water content.[53]

Apart from hormonal factors, uterine contractions can also alter the spaces between these macromolecules by mechanical pressure. Furthermore, these mechanical forces contribute to the orientation of the mucin filaments. It is suggested that the outward flow of the cervical mucus establishes a linear alignment of parallel strands, creating aqueous channels between the filaments that direct sperm upward.[2,7,58] Given this longitudinal orientation, with mucus outflow originating in the crypts of the cervical

Table 1 - Factors affecting fertility at the vaginal and cervical levels

Vagina	Increase in the vaginal pH: alteration in the vaginal flora, leading to an increased susceptibility to infectious processes (phagocytosis of sperm, pro-inflammatory cytokines)
	Seminal plasma deficiency in neutralizing the vaginal pH: hypospermia, deficient seminal vesicle secretion
	Deficiency in semen liquefaction (e.g., abnormal prostatic secretions)
	Use of lubricants toxic to sperm
Cervix	Previous surgery (e.g., cauterization, cone biopsy, curettage)
	Infections
	Müllerian abnormalities
	Exogenous: intra-uterus diethylstilbestrol (DES)
Cervical Mucus	Hormonal: abnormal estrogen levels and premature progesterone rise
	Inflammatory: chronic cervicitis/acute inflammatory conditions
	Genetics: cystic fibrosis
	Exogenous: clomiphene citrate, propranolol, nicotine
	Trace elements: excess levels of copper, iron and selenium
	Male-related: asthenozoospermia and abnormal sperm morphology
	Immunological: antisperm antibodies in the female serum and semen

epithelium, it has been postulated that spermatozoa are constrained to swim in the direction of least resistance, that is, along the tracts of mucus outflow in the direction of the cervical crypts.[82,83] This theory is in agreement with the notion that spermatozoa entering the cervix are directed toward the cervical crypts, which are the sites of mucus secretion that serve as possible sperm storage reservoir. The number of spermatozoa within the cervical mucus is relatively constant for the first 24 hours after coitus. Spermatozoa may retain their fertilizing capacity in the human cervical mucus for up to 48 hours and their motility for as long as 120 hours after ejaculation. However, the number of motile sperm within the mucus is markedly decreased after 48 hours.[84–86] From their temporary storage location within the cervical crypts, sperm can be released gradually over time, thus enhancing the probability of fertilization. As the size of the interstices is usually smaller than the size of sperm heads, spermatozoa must actively push their way through the mucus. Therefore, one cause of infertility, presumably, is the reduced progressive movement of sperm that prevents sperm movement through the mucus.[2,7]

It is generally believed that another potentially important feature of human cervical mucus is its ability to restrict migration of abnormal spermatozoa, thus acting as a "filter" that eliminates deficient sperm.[21,87,88] It has been shown that abnormal sperm have a poorer hydrodynamic profile compared with morphologically normal motile sperm.[7,21,87,88] Moreover, sperm movement is probably influenced by the interaction between the mucus and the surface properties of the sperm head; for instance, sperm antibodies on the sperm head may inhibit sperm movement through the mucus.[89]

Like the vagina, the cervix can assemble immune responses. Studies have shown that vaginal insemination stimulates the migration of leukocytes, particularly neutrophils and macrophages, into the cervix as well as into the vagina.[90,91] This leukocytic invasion protects against microbes that are often seen in the semen, but it does not represent a barrier to normal sperm under physiological conditions.[88] On the other hand, it has been demonstrated that neutrophils bind to and ingest human sperm if the female serum contains both serological complement and complement-fixing antisperm antibodies.[92] This process occurs when the female becomes immunized against sperm antigens. As already mentioned, immunoglobulins, mainly IgG and IgA, have been detected in human cervical mucus. Secretory IgA is produced locally by plasma cells in subepithelial connective tissue. Although immunoglobulins provide protection from microorganisms, immunological infertility can occur when antibodies present in the cervical mucus recognize sperm-bound antigens.[93] Since complement proteins are present in the cervical mucus, antibody-mediated sperm destruction as well as leukocytic sperm capture may occur.[94] Despite the fact that not all antisperm antibodies are complement-activated, they can still interfere with sperm progression by attaching the sperm head and avoiding spermatozoa to enter the microarchitecture of the cervical mucus network.[93,95] Furthermore, the presence of ASA in the male can also result in infertility since such antibodies have been shown to affect sperm function.[22,23,36,96]

CONCLUSIONS

Among the several conditions that may be involved in the pathophysiology of unexplained infertility at the vaginal and cervical levels, physicians should pay particular attention to (i) inadequate buffering capacity of acid vaginal pH, (ii) alterations in cervical anatomy caused by surgeries, birth defects and infections, (iii) alterations in the cervical mucus caused by hormonal dysfunctions, inflammatory disorders, cystic fibrosis, exogenous and immunological factors.

SOBRE O PAPEL DO MUCO CERVICAL E DO PH VAGINAL NA GÊNESE DA INFERTILIDADE INEXPLICADA

RESUMO

O diagnóstico de infertilidade inexplicada baseia-se na presença de espermograma normal, constatadas também permeabilidade tubária e função ovulatória normais. Entre as várias causas potenciais de infertilidade inexplicada, a presença de muco cervical e pH vaginal anormais devem ser consideradas. Embora a produção adequada de muco cervical seja essencial para o transporte dos espermatozóides da vagina para a cavidade uterina, e tanto o canal vaginal quanto o colo do útero desempenham função importante como barreira à passagem dos espermatozóides, estes fatores recebem pouca atenção na investigação de casais com infertilidade inexplicada. Uma redução substancial do número de espermatozoides ocorre à medida que estes percorrem o trato reprodutivo feminino. Partindo de cerca de 200 a 300 milhões de espermatozoides depositados na vagina, apenas algumas centenas alcançam a proximidade do oócito. Alterações do muco cervical podem rapidamente transformar o colo do útero num ambiente hostil, que em conjunto com alterações no ambiente vaginal e da estrutura de colo do útero, podem apresentar-se condições impedientes para a concepção natural; desse modo, convertem-se em causa de infertilidade. Nesta revisão, discutimos o papel fisiológico do pH vaginal e do muco cervical na fertilidade, descrever várias condições que podem tornar o muco cervical hostil aos espermatozoides e, por fim analisamos como estes fatores interferem na fisiopatologia da infertilidade inexplicada.

UNITERMOS: transporte espermático; muco cervical; concentração hidrogeniônica; aglutinação espermática; moléstia cervical; moléstia vaginal; infertilidade feminina; infertilidade inexplicada.

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