The role of the cervix in fertility: is it time for a reappraisal?

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Abstract: Knowledge regarding the role of the cervix in fertility has expanded considerably over the past 20 years and in this article, we propose that it is now time for its function to be reappraised. First, we review the anatomy of the cervix and the vaginal ecosystem that it inhabits. Then, we examine the physiology and the role of the cervical mucus. The ongoing mystery of the exact mechanism of the sperm—cervical mucus interaction is reviewed and the key players that may unlock this mystery in the future are discussed. The soluble and cellular biomarkers of the lower female genital tract which are slowly being defined by contemporary research are reviewed. Attempts to standardize these markers, in this milieu, are hindered by the changes that may be attributed to endogenous or exogenous factors such as: age, hormonal changes during the menstrual cycle, ectropion, infection, smoking and exposure to semen during sexual intercourse. We review what is known about the immunology of the cervix. With the widespread use of large loop excision of the transformation zone (LLETZ) for treatment of cervical intraepithelial neoplasia, the anatomy of the cervix is changing for many women. While LLETZ surgery has had very positive effects in the fight against cervical cancer, we debate the impact it could have on a woman’s fertility.

Key words: cervix / fertility / sperm—cervical mucus interaction / lower female genital tract / large loop excision of the transformation zone

Introduction

The cervix plays a fascinating gatekeeping role in first, preventing the ascent of pathogens from the vagina into the uterus, and second, allowing the ascent of sperm to the Fallopian tubes. It is also crucial for the maintenance of pregnancy in the uterus until the onset of labour. This review focuses on the important fertility-related functions of the cervix and in particular the sperm—cervical mucus interaction.

When semen is deposited in the female reproductive tract during sexual intercourse, the first barrier sperm must surmount en route to the Fallopian tubes is the cervix and its mucus. Commonly cited causes of subfertility in women include anovulation, tubal disease, endometriosis, male factor and unexplained subfertility, with often scant attention paid to the frequently forgotten cervical factor. It is estimated that abnormalities of cervical secretions may account for ≏3% (Hull et al., 1985) to ≏8% (Brandes et al., 2010) of cases of infertility in women.

Intrauterine insemination (IUI), IVF and ICSI, treatments which bypass the cervix and its mucus, have drawn attention away from the function of the cervix and its interaction with sperm. More recently, however, lower female genital tract soluble and cellular biomarkers are being defined (Kyong et al., 2012) and newer methods of analysing sperm dysfunction in men are being uncovered (Evenson and Wixon 2008; Hwang et al., 2011a,b). Additionally, the advent and success of cervical screening programmes (Giorgi Rossi and Ronco 2013), with increased use of large loop excision of the transformation zone (LLETZ) treatment in young women (Kyrgiou et al., 2006) and our increasing knowledge of the prevalence of human papilloma virus (HPV; Castle et al., 2011), we contend that attention needs to be re-focused on the function of the cervix, its secretions and the interactions these have with sperm.

Our paper starts with a review of the anatomy and physiology of the cervix and cervical mucus. We then describe components of the sperm—cervical mucus interaction and look at recent advances in our understanding of the complex relationships at this level. We also review the inflammatory and immunological factors of the cervix. Lastly, we examine the possible impact that surgery, mainly in the form of LLETZ, may have on the cervix and its vital functions.

Anatomy and physiology of the cervix

The cervix is made up of the ectocervix and endocervix and is on average 3–4 cm long and 2.5 cm wide (Pardo et al., 2003; Mazouni et al., 2005; Robert et al., 2013). The ectocervix is the portion of the cervix projecting into the vagina. It is composed of non-keratinized stratified squamous epithelium and is divided into anterior and posterior lips. The squamo-columnar junction is the area where the epithelial cells of the endocervix and ectocervix meet (Herfs et al., 2013). In this area, the columnar cells of
the endocervix undergo metaplasia to the squamous cells of the ectocervix. The opening of the ectocervix to the vagina is called the external os. Although studies of cervical length in non-pregnant women are few, it is acknowledged that the size and shape of both the cervix and the external os differ in women (Pardo et al., 2003; Mazouni et al., 2005) and vary with age, hormonal changes, parity and surgical treatments to the cervix.

The passageway between the external os and the uterine cavity is referred to as the endocervical canal. This canal is ~7 mm in width but again this can vary with age and parity. The endocervical canal terminates at the internal os, which is the opening of the cervix to the uterine cavity. The stroma of the cervix is made up of dense, fibro-muscular tissue which in turn is made up of collagenous connective tissue, smooth muscle and elastic tissue and ground substance—glycosaminoglycans. Through this matrix pass lymphatic vessels and nerves. The columnar epithelium of the endocervical canal comprises a single layer of mucin-secreting cells (Moghissi et al., 1976a,b) which produce abundant secretory products which are mostly mucin glycoproteins.

Cervical and vaginal ecosystem

The vaginal ecosystem is complex and complements the role of the cervix in balancing the conflicting need for protection against infection but also entry of sperm to the upper genital tract. The vaginal pH is kept between 3.8 and 4.5 by the anaerobic metabolism of vaginal glyco- gen to acid products, predominantly acetic and lactic acid (Boskey et al., 1999) and this low pH is toxic to both bacteria and sperm. The vagina is colonized by a large quantity of micro-organisms with the Lactobacillus spp. predominating. It is thought that it is the ability of the lactobacilli to produce hydrogen peroxide, which can combine with chloride in the vagina, that produces this acidic antimicrobial defence (Klebanoff et al., 2002). L. crispatus, the most common species found in the female vagina are able to tolerate and grow in these acid conditions (Wilks et al., 2004). These lactobacilli form an important non-specific antimicrobial host defence which, combined with the vagina’s low pH, protects the lower female genital tract from ascending infections (Carr et al., 1998).

In contrast to the vagina, the pH of cervical mucus is ~7.0 (Correa et al., 2001) and so is considerably less acidic. The normal pH of semen ranges from 7.2 to 8.0 (Haugen and Grotmol, 1998) and thus the cervical mucus pH supports sperm viability (Eggert-Kruse et al., 1993).

Cervical mucus

The cervical mucosal secretions fill the opening of the cervical canal and contribute significantly to the dual roles of the cervix in preventing the ascent of pathogens but also facilitating the ascent of sperm to the Fallopian tubes. The cervical mucus is composed of water (95—99%), ions, enzymes, bactericidal proteins, plasma proteins and mucins.

Our understanding of the biochemical properties of cervical mucus is still evolving. Mucins are large polymeric molecules that contribute to the gel-like property of mucus. They are glycosylated proteins and are complicated to characterize due to their large size, polymeric nature and heterogeneous glycosylation (Gilks et al., 1989; Argueso et al., 2002; Andersch-Bjorkman et al., 2007). Recent studies have examined the different types of mucins and how they vary over a woman’s cycle. Up to 16 different mucins have been identified with at least five in the female reproductive epithelia (Andersch-Bjorkman et al., 2007). Three have been described as gel forming (MUC 5B, MUC 5AC, MUC 6) and two are membrane bound (MUC1 and MUC 16). It appears that MUC5B is the major mucin of the cervical mucus and that it has its highest expression at midcycle (Gipson et al., 1997, 2001; Flori et al., 2007).

Sperm—cervical mucus interactions

It has long been recognized that the sperm cervical mucus interactions are important for fertility. The post-coital test (PCT), described first by Sims in 1866, examines the interaction between sperm and cervical mucus (Kremer and Kroeks 1975; Farhi et al., 1995). The PCT is performed close to ovulation—the couple are asked to have sexual intercourse and several hours later the woman’s cervical mucus is examined for the presence of active sperm. If active sperm are found, the PCT is
deemed positive or normal and the couple are, unlikely to have an abnormal interaction between cervical mucus and sperm. The PCT has been used to predict the likelihood of natural conception (Snick et al., 1997; Hull and Evers 1999; Hunault et al., 2004), with best discriminating results found when the duration of infertility was < 3 years (Glazener et al., 2000). The authors concluded that if a couple had an abnormal or negative PCT they could be referred for assisted reproduction treatment, even if they had infertility for < 3 years, but if they had a normal or positive test they could continue trying to conceive with a high chance of success.

The PCT has, however, been criticized (Oei et al., 1995), as it can be affected by poor coital technique, ovalutory problems, poor timing of ovulation and immune factors. The test is also difficult to organize and time appropriately, and may be deemed relatively unpleasant by some couples. With the advent of IUI and IVF, procedures which bypass the cervical mucus and its functions, the PCT has, in recent years, been largely abandoned in most centres.

The penetration of spermatozoa into human cervical mucus has been used to provide important predictive information about sperm function (Barratt et al., 1989, 1992). The range of human sperm longevity is thought to vary from 24–48 h to as long as 5 days (Zinaman et al., 1989). Zinaman et al. in their paper in 1989 reported that there was prolonged acrosomal integrity of sperm recovered from the cervix of women post- artificial insemination for up to 3 days in vivo and concluded that those findings supported the hypothesis that sperm function is conserved in the cervix and that the cervix may act as a site of sperm storage in women (Zinaman et al., 1989).

As foreign cells, sperm are at risk of phagocytosis involving leucocytes, antibodies and complement in the female reproductive tract. Like all cell surfaces, sperm are coated with thick glyocalyx formed by a complex array of glycans, the oligo and polysaccharides attached to glycoproteins and glycolipids. This sperm glyocalyx is rich in sialic acids (Schroter et al., 1999). Sialic acids are a diverse family of nine carbon sugars that are mostly derived from N-acetylneuraminic acid (Neu5Ac). They are typically found attached to other sugars or another sialic acid molecule. Sialic acids can be found attached to glycoproteins, as in the case of cervical mucus. It is thought that while they therefore contribute to the heterogeneity of glycoproteins, they also act as ligands in complex cellular recognition events (Crocker 2002).

The sialic acid content of mammalian sperm has been shown to correlate positively with protection from phagocytosis and therefore appropriate sialylation of sperm is important if it is to survive in the epididymis and in the female genital tract (Toshimori et al., 1991; Lassalle and Testart 1994; Velasquez et al., 2007). Another component of the sperm surface glycoalyx is the protein β-defensin 126. Tollner et al., in 2008, reported that in the macaque monkey, the protein β-defensin 126 (DEFB126), which coats the macaque sperm, was crucial for sperm to penetrate and move efficiently in peri-ovulatory mucus (Tollner, Yudin et al., 2008). Sialic acids on the sperm surface also play a role in attachment of the sperm to the human oviduct, thus facilitating sperm transport through the female genital tract (Tollner et al., 2011). Tollner et al. went on to report that sperm from men who carried a genetic variant of this sperm surface protein DEFB126 exhibited reduced ability to penetrate hyaluronic acid (mucus substitute) were less likely to get their partners pregnant and took longer to achieve a live birth (Tollner et al., 2011, 2012).

While sialic acids are important for sperm binding to mucus and the oviduct, sialic acid content has been shown to be negatively correlated with the capacity of the sperm to bind to the zona pellucida of the ovum (Toshimori et al., 1991; Lassalle and Testart 1994; Velasquez et al., 2007). Sperm are not able to fertilize eggs immediately after ejaculation. It is known that sperm acquire fertilization capacity by residing in the female genital tract and undergoing a series of physiological changes, which are collectively known as capacitation. Interesting data are accumulating regarding possible mechanisms by which sperm achieve capacitation in the female reproductive system. Focarelli et al. hypothesized and demonstrated in 1990 that the removal of glycoconjugates from the sperm surface in vitro by using human albumin played a role in achieving capacitation (Focarelli et al., 1990). This group then went on to describe how there is an increase in sialidase activity in female cervical mucus around ovulation and that this could be involved in modifying the rheological properties of mucus to favour sperm progression at ovulation (Flori et al., 2007) by removing some of the sialic acids. Andersch-Bjorkman et al. also reported in 2007, that the differences in cervical mucus during ovulation appeared to be due to changed mucin glycosylation, attesting that cervical mucus could be promoting sperm penetration by its low sialic acid content but the exact mechanism of how this helped sperm enter was still not understood (Andersch-Bjorkman et al., 2007).

A recent in vitro study of capacitation in mouse and human sperm demonstrated the release of two sialidases from sperm during capacitation. Inhibition of these sialidases interfered with sperm binding to the zona pellucida and a lack of one or both of these sialidases in some male sperm was associated with idiopathic male infertility (Ma et al., 2012).

Receptors for sialic acids have been recently described and are termed Siglecs: Sialic acid-binding immunoglobulin-like lectins. These receptors are a relatively recent discovery and have been implicated in cell–cell interactions in the haemopoietic, immune and nervous systems (Crocker et al., 2007). Siglecs are found on leukocytes, and it has been proposed that they may play a role in sperm survival in the female genital tract (Ma et al., 2012).

The discovery of the relationship between the β-defensin 126 mutation and fertility, combined with our increasing knowledge of changes in glycosylation and the sialic acid content of sperm and cervical mucus, is bringing us ever closer to determining the exact mechanism of the sperm–mucus interaction. We contest that as this knowledge develops, it may be worth re-evaluating the benefits of a clinical test to assess the sperm–cervical mucus interaction in couples.

**Inflammatory and immunological factors at the cervix**

As with other cervical factors, the function of the immune system at the cervix is 3-fold—to protect the genital tract against infections, to allow sperm entry for fertilization to occur and to protect the fetus from infection during pregnancy. The mucosal immune system of the cervix consists of immune cells that migrate there, as well as resident epithelial cells and supportive stromal cells (Wira et al., 2005). The epithelial cells are sentinel cells that function as part of the innate and adaptive immune system. They are responsive to hormonal changes and produce a number of soluble mediators. It is these soluble mediators that influence immune cell migration and defense against local pathogens, while also optimizing reproductive potential (Wira et al., 2010).

Given that sperm are foreign bodies in the female reproductive tract, immune mechanisms are involved in allowing these allogeneic cells to
remain in the female genital tract without eliciting an adverse immune response. We have already described the sialic acid rich coating on the sperm, and their corresponding receptors, Siglecs. These are thought to have an important role in protecting sperm from the female’s immune response after sexual intercourse (Crocker et al., 2007). It is thought that the sperm must properly engage with the Siglecs to prevent female immune recognition and avoid demise (Ma et al., 2012).

Cellular immunity plays an important role in the early phase of infection, with cytokines (small signalling proteins secreted primarily by immune cells), playing a significant role in the defence against infections, especially the very common HPV with its high risk types that are associated with the development of cervical cancer (Govan et al., 2006; Song et al., 2007).

Attempts to standardize cytokine measurements in cervical mucus are hindered by changes that could be attributed to endogenous or exogenous factors such as: age, hormonal changes during the menstrual cycle, the presence of ectropion, infection, smoking and exposure to semen during sexual intercourse (Kyongo et al., 2012). Indeed, most studies regarding cytokines in the cervix have come either from those investigating the immune response to infections, such as human immunodeficiency virus and HPV, and its role in the development of neoplasia and cancer (Song et al., 2007; Hwang et al., 2011a,b; Ali et al., 2012; Mosaffa et al., 2012) or studies that have investigated the relation between inflammation of the cervix and preterm labour. Optimal methods for sample collection of these soluble mediators also vary, with endocervical swabs and cervicovaginal lavage (CVL) reported to be best (Dezzutti et al., 2011).

Fewer studies have investigated how the immune system of the cervix could impact on fertility. After sperm insemination in the female reproductive tract, a humoral and cellular immune response is mounted by the uterine cervix to instinctively protect the female from anticipated infection. Older studies reported a leukocytic reaction in the female reproductive tract in response to sperm (Pandya and Cohen 1985; Thompson et al., 1992). Many different cytokines have been studied—interleukin (IL)-1β, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, macrophage inflammatory protein (MIP-1α), granulocyte colony-stimulation factor, RANTES, tumour necrosis factor, IFN (interferon)-gamma (Kanai et al., 1997; Gargiulo et al., 2004; Lieberman et al., 2008; Hwang et al., 2011a,b), to name a but a few, with IL-1α, IL-6 and IL-8 being found in highest concentrations. Increases in IL-8 in response to seminal plasma are purported to produce an increase in the neutrophil population after insemination (Thompson et al., 1992) which would ensure the rapid demise of sperm after insemination.

Other studies report that IL-6, IL-8 and endogenously produced antimicrobials fall significantly at midcycle and remain low until just before menstruation (Keller et al., 2007; Wira et al., 2010), supporting the hypothesis that the immune system, under hormonal regulation, optimizes conditions for pregnancy to be achieved. The cervical immune system needs to be able to distinguish between pathogens which could be harmful and allogeneic sperm that are necessary for reproduction. Around ovulation, it is suggested that this may open a window of vulnerability for sexually transmitted infection in the female (Nardelli-Haesiger et al., 2003).

Natz et al. (1995) reported significantly higher levels of IFN-γ in women with idiopathic infertility compared with fertile controls. Gargiulo et al. (2004) investigated implantation-related cytokines in the cervicovaginal secretions and peripheral blood of fertile women during ovulatory menstrual cycles. While they found no correlation between levels of cytokines in CVL and serum, they concluded that macrophage-colony stimulating factor (M-CSF) and epidermal growth factor (EGF) warranted further study in fertile and infertile women to determine their predictive values as minimally invasive markers of uterine receptivity. This was due to the fact that they were measurable in the CVL samples of all their study subjects, that M-CSF levels were correlated with serum estrogen levels and that CVL EGF levels correlated with endometrial EGF mRNA levels during the mid-secretory phase of the cycle.

In summary, ongoing research to define the exact role that inflammatory mediators play in the dynamic environment of the lower female reproductive system is challenging. However, the hormonal changes observed and the previously mentioned allogeneic nature of sperm, suggest that further study may be informative.

Large loop excision of the transformation zone

LLETZ or Loop Electrosurgical Excision Procedure (LEEP) has been in use for almost 25 years (Prendiville et al., 1989) and is now the most commonly employed treatment option for cervical intra-epithelial neoplasia (CIN; Kyrgiou et al., 2006). Several studies and meta-analyses have looked at the links between LLETZ treatment and obstetric outcomes, particularly preterm birth (Sadler et al., 2004; Kyrgiou et al., 2006; Albrechtsen et al., 2008; Noehr et al., 2009; Bruinsma and Quinn 2011; Castanon et al., 2012; Poon et al., 2012), but studies investigating the potential fertility consequences of such a common procedure have been surprisingly lacking.

In relation to the LLETZ procedure, there are three potential mechanisms by which it could impact on fertility (Bigrigg et al., 1994): Cervical stenosis, secondary infection or changes in the physical characteristics of cervical mucus.

Kennedy et al. (1993) raised the possibility of cervical stenosis and amucorrhoea following treatment for CIN in their retrospective study in 1993. They found that two women out of 15 had cervical stenosis, one following a LLETZ and one following a LLETZ cone. Bigrigg et al. (1994) performed a case-controlled questionnaire involving 250 matched pairs and found no apparent difference in infertility between the groups. This study was limited by size and length of follow-up post-LLETZ. Cruickshank et al. (1995) followed up 1000 women post-LLETZ with a questionnaire and found that none of the women subsequently investigated for infertility were found to have cervical stenosis or amucorrhoea. Turlington et al. in 1996 (Turlington et al., 1996), in their small case-control study (n = 111) found that LLETZ treatment did not cause a significant detrimental effect on fertility.

Most recently, in (2013), Spracklen et al. reported that women with a history of cervical treatment for CIN took longer to conceive when compared with women who had no treatment or colposcopy only (Spracklen et al., 2013). However, their treatment groups were small and were made up of a varied group of treatments—46 cone biopsies, 45 LEEP, 44 cryosurgeries and 17 laser vaporizations. The study only included women who delivered preterm or had small for gestational age babies. The time to conceive was assessed as <12 months or >12 months and no information was provided regarding fertility history.

With regard to LLETZ treatments and LLETZ specimens, it is important to remember that they are a very heterogeneous group (Arbyn et al., 2008; Phadnis et al., 2010). Specimens from LLETZ treatments vary...
considerably in size and it has been confirmed that the height (or depth) and volume of the specimens can predict the relative risk of pregnancy-related morbidity (Leiman et al., 1980; Sadler et al., 2004; Acharya et al., 2005; Khalid et al., 2012). Whether this size effect also relates to fertility and the impact it could have on the cervix, such as the development of stenosis or cervical mucus production, has yet to be determined. Similarly, the effect of multiple LLETZ procedures and of more invasive therapy, such as cone biopsy, requires evaluation.

In summary, studies to date regarding the effect of cervical surgery on fertility are limited by small sample size, short duration of follow-up and insufficient detail on the type and extent of surgery.

Conclusion
The cervix is the door to the female reproductive system. Anatomically, it must allow sperm passage into the uterus through the endocervical canal and it must produce mucus to facilitate sperm entry. Immunologically, it must recognize and prevent pathogen entry. Further research is needed to definitively describe the likely inflammatory mediators that are in control, or in flux, at the cervical mucosal level. With regard to the exact sperm cervical mucus interaction at the molecular level, fascinating information is accumulating regarding the sperm surface glycoalyx and sialic acids and their Siglec receptors in sperm and mucus. Interesting genetic variants of sperm surface proteins may also be important. Further research is also needed into this sperm–cervical mucus interaction which may lead to clinical applications, not only in fertility treatment but also in new avenues for contraception. Finally, over the last 20 years surgery to the cervix remains the principal anatomical change but also in new avenues for contraception. Finally, over the last 20 years surgery to the cervix remains the principal anatomical change and the impact it could have on the cervix, such as the development of stenosis or cervical mucus production, has yet to be determined.

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