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Review

Epithelial ion channels in the regulation of female reproductive tract fluid microenvironment: implications in fertility and infertility

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Abstract: An optimal fluid microenvironment in the female reproductive tract is considered to be crucial for successful reproductive events. Fluid absorption and secretion across the reproductive tract epithelia largely depends on electrolyte transport through the apically and basolaterally located ion channels, working together with an array of other transporters. This review will discuss the role of epithelial sodium channel (ENaC) and the cystic fibrosis transmembrane conductance regulator (CFTR) in regulating the fluid volume and composition of the reproductive tract and their importance in various reproductive events such as sperm capacitation and implantation. Disturbance of the fluid microenvironment due to defects or abnormal regulation of these ion channels as causes for a number of pathological conditions, such as ovarian hyperstimulation syndromes, hydrosalpinx and infertility, is also discussed.

Key words: cystic fibrosis transmembrane conductance regulator; epithelial sodium channel; epithelial cells; sperm capacitation; implantation; infection

上皮细胞离子通道对雌性生殖道内液体微环境的调节作用：对生殖与不孕的影响

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摘 要：雌性生殖道内适宜的液体微环境对一系列生殖事件起至关重要的作用。位于生殖道上皮细胞顶膜或基底膜的一系列离子通道和转运体，通过对水、电解质的跨膜转运，从而调节雌性生殖道内液体的分泌与吸收。本综述着重探讨了上皮细胞钠离子通道和囊性纤维化跨膜电导调节体对雌性生殖道内液体容量和成分的调节以及它们在不同生殖事件，比如精子获能及着床中的重要作用。同时对因离子通道失活或失调引起的雌性生殖道内液体微环境稳态失衡导致的一系列病理改变，如卵巢过度刺激综合征、输卵管积水以及不孕提出了新的见解。

关键词：囊性纤维化跨膜电导调节体；上皮细胞钠离子通道；上皮细胞；精子获能；着床；感染
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1 Introduction

The epithelial layer covering the inner surface of the female reproductive tract plays an important role in reproductive physiology by providing an optimal fluid microenvironment necessary for the success of various reproductive events occurring along the genital tract, such as sperm capacitation, fertilization, embryo development and implantation. After ejaculation, sperm enter into the female genital tract through the cervix and uterus, swim upward to the Fallopian tube (oviduct) where they meet and fertilize the egg. The tubal epithelium secrets/transports electrolytes, nutrients and micro molecules, oviduct specific glycoproteins, and also maintains adequate pH and

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osmolality needed for both sperm and oocyte. Sperm stay in the lumen for hours to days prior to fertilization and must be maintained in a viable state during this period. This is also true for the embryos undergoing early embryo development in the tubes. It also provides a suitable fluid microenvironment that keeps sperm motile while the sperm go through the uterine cavity towards the oviduct with the aid of beating of uterine cilia[1]. In this process, the sperm gain the capacity for fertilizing an egg, a process termed capacitation, which proved to largely depend on the components of the uterine/oviductal fluid. The luminal fluid also conveys a variety of signals between the endometrium and the blastocyst; meanwhile they provide a compatible ambient for the blastocyst growth. In the process of blastocyst implantation, the volume and composition of uterine fluid undergo significant changes for blastocyst attachment and embedding[2]. Disturbance of the fluid microenvironment, i.e., by bacterial infection or in diseases such as cystic fibrosis (CF), is known to result in infertility although the underlying causes remain largely unknown.

It is unquestionable that the fluid microenvironment in the female genital tract is important for reproduction, as highlighted by the inability of sperm to fertilize the egg until they undergo the process of capacitation in the female reproductive tract. Although attempts and observations were made as early as 80 years ago, showing dynamic changes in uterine fluid volume as well as vast difference in uterine and oviductal fluid compositions as compared to that in the blood[3], the cellular mechanisms underlying the fluid formation along the tract, as well as the regulatory mechanisms governing the dynamic and cyclic changes in the fluid volume and composition, remained unknown for quite a long time. In the last decade, our laboratory has undertaken a series of studies on rodent models to investigate the mechanisms of electrolyte transport across the epithelia in the female reproductive tract and to understand its changes and regulation under various physiological and pathological conditions. Some of the details of our studies have been reviewed elsewhere[4,5]. In this review, we will highlight some of our findings and discuss possible mechanisms underlying physiological changes and disorders or diseases in the female reproductive tract in light of these findings.

2 Cystic fibrosis transmembrane conductance regulator (CFTR) and epithelial sodium channel (ENaC) expression profile and functional implications in reproductive events

2.1 Regional difference and cyclic changes in CFTR and ENaC expression

The female reproductive system can be viewed as a continuous tract with the vagina, cervix and uterus situated at the lower end and the ovaries and Fallopian tubes (or oviducts) in the upper part. The luminal fluid can flow continuously throughout the tract, at least during some period of time in the cycle, although regional differences, in terms of volume and composition, have been noted. It had been long held that the fluid in the female reproductive tract was originated from the upper part of the tract, particularly the oviducts, however, whether other parts of the genital tract, such as the uterus, might have secretary activities of their own were in question. To answer this question, Chan *et al*. established a primary culture model of mouse endometrial epithelium, which could be assessed by the short-circuit current (I_{SC}) measurement and a number of other techniques to determine its ion transport and related cellular mechanisms[6]. The established mouse endometrial epithelial culture appears to exhibit predominantly Na^+ absorptive characteristic under unstimulated condition; e.g., the basal current was largely inhibited by apical replacement of Na^+ or a blocker shown to inhibit ENaC. The Na^+ absorptive characteristic under unstimulated condition has also been observed in cultured human endometrial epithelium[7]. The cultured mouse endometrial epithelium also exhibits predominant anion (both Cl^- and HCO_3^-) secretory activities upon stimulation with various secretagogues. While transient responses can be induced by ATP (10 μmol/L, apical), Arg^8 vasopressin (1 μmol/L, basolateral), VIP (1 μmol/L, basolateral), and bombesin (10 μmol/L, basolateral), more sustained increase in the current response could be induced by adrenaline (1 μmol/L, basolateral) or forskolin (10 μmol/L). The later was further demonstrated to be mediated by the cAMP-dependent pathway, involving possible activation of CFTR[8]. Indeed, the cultured endometrial epithelial cells were shown to have protein and functional expression of CFTR, as demonstrated by Western blot and the whole-cell voltage-clamp recording, respectively[9]. These results indicated that the uterine epithelium has its own ion transport mechanisms, such as ENaC and CFTR for Na^+ absorption and anion secretion, respectively. Since the flow of fluid follows the movement of electrolytes, we speculated that the variation in CFTR and ENaC expression in different regions of the genital tract could be the underlying mechanism responsible for the observed regional difference in absorptive/secretory activities and thus fluid volume and composition. Also, if the expression of these ion channels could be regulated by

ovarian hormones, cyclic changes in fluid volume and composition would be anticipated.

Chan *et al*.[10] undertook an *in situ* hybridization study to examine CFTR and ENaC expression along the entire female genital tract of the mouse at different stages of the estrus cycle and the results provided for the first time a possible molecular basis for fluid formation in different regions of the female reproductive tract. The *in situ* hybridization study revealed abundant expression of CFTR in the ovary and oviduct throughout the estrus cycle, suggesting that this part of the female reproductive tract plays a major secretory role and is the constant source of NaCl and fluid in the genital tract. The lack of ENaC expression in the ovary and oviduct suggests that little Na^+ and fluid reabsorption occurs in this part, and thus, the fluid found in the lower part of the genital tract may be contributed, at least partly, by the fluids of ovarian or ductal origin. On the contrary, the expression of all ENaC subunits in the mouse cervical and vaginal epithelia throughout the estrus cycle indicates a primarily reabsorptive role of the cervix and vagina with ENaC as a main pathway for Na^+ reaborption, which eventually leads to water reabsorption, to prevent salt and water loss from cervical and vaginal discharges. The molecular evidence for polarized mRNA expression of CFTR and ENaC at the two ends of the genital tract agrees well with the findings from previous electrophysiological studies. A transvaginal potential difference with negative luminal polarity consistently observed in women throughout the menstrual cycle[11], together with a luminal NaCl concentration lower than that in the plasma[12], is consistent with the expression of ENaC in the vagina and cervix. A lack of ENaC expression in the oviduct may account for the relatively small and amiloride-insensitive basal transepithelial potential difference and I_{SC} observed in primary cultures of oviduct epithelia of rabbit[13], mouse[14] and human[15].

The observed estrus cycle-dependent expression of CFTR, i.e., at proestrus in the vagina and cervix, and at estrus in the uterus, is consistent with the cyclic profile of ovarian hormones[16]. Stimulation of CFTR expression by estrogen both *in vivo* and *in vitro*[17,18] has been demonstrated and downregulation of CFTR by progesterone has also been reported[19]. The ovarian hormone-dependent regulation of CFTR expression provides a physiological basis for the cyclic changes in fluid volume, which corresponds to the highest fluid accumulation during proestrus and estrus and a minimum at diestrus[3]. The observed out-of-phase co-expression of CFTR and ENaC in the uterus, i.e., high expression of CFTR but low ENaC expression at estrus and low CFTR expression but high ENaC expression at diestrus, provides further explanation for the observed cyclic changes in uterine fluid volume. The difference in the expression of these two ion channels appears to be due to their difference in response to estrogen, which stimulates CFTR expression but downregulates ENaC expression, as demonstrated by the I_{SC} measurements showing decreased Na^+ absorption and increased Cl^- secretion in estrogen-treated endometrial epithelia[10]. Thus, this may explain maximal fluid secretion during the early phase of the estrus cycle, when the level of estrogen is the highest. Similarly, downregulation of CFTR to slow down fluid production and upregulation of ENaC to increase the rate of reabsorption may be accounted for the disappearance of uterine fluid observed at diestrus. The negligible expression of CFTR at diestrus may further augment ENaC function to facilitate fluid reabsorption, since CFTR has also been shown to act as a negative regulator of ENaC in mouse endometrial epithelial cells[20]. The out-of-phase co-expression of CFTR and ENaC in the uterus may be of physiological significance. While maximal CFTR expression at estrus may enable a higher rate of uterine fluid production to facilitate sperm transport and sperm capacitation (see below), downregulation of CFTR and upregulation of ENaC at metestrus and diestrus may reduce the fluid volume in the lumen to enhance close contact between the endometrial surface to facilitate implantation of the embryo.

The expression pattern of ENaC and CFTR in the cervix and vagina suggested that both ENaC and CFTR work closely together to maintain an optimal cervical and vaginal fluid microenvironment for sperm movement and optimal antimicrobial activity. In addition to a primarily reabsorptive role of the vagina and cervix as dictated by the expression of ENaC throughout the cycle, the expression of CFTR at proestrus may serve to lubricate the cervical and vaginal lumen and reduce the viscosity of the mucus for sperm movement towards the oviduct for successful fertilization. In fact, the primary cause for the reduced fertility rate observed in CF women has been suggested to be the formation of thick cervical mucus, which acts as a barrier to sperm penetration[21]. Taken together, the differential expression of CFTR and ENaC in different regions of the genital tract and at different stages of the cycle appears to be the basis for the observed regional difference and cyclic changes in fluid volume and composition along the female reproductive tract, which are important for various reproductive events (see section below).

2.2 Expression of CFTR and ENaC during implantation

Implantation is a complex and dynamic process that is initiated by the adhesion between the embryonic trophectoderm and the epithelial cells of the uterine endometrium. This rate-limiting process in reproduction remains a poorly understood phenomenon, but it is thought to be greatly influenced by the uterine fluid. The disappearance of luminal fluid in the pre-implantation period, a characteristic feature of many species, is considered to be a mechanism leading to the "closure" of the lumen, thereby enabling embryos to be held in contact with uterine epithelium before initiation of implantation. Yang *et al*.[22] observed differential expression of ENaC and CFTR during pre-implantation in mice. In that study, ENaC mRNA expression correlated well with its immunoreactivity, showing a maximal level on day 3, the day before implantation (day 4), and a gradual decline in expression afterwards. The upregulation of ENaC on day 3 may result in enhanced Na^+ and fluid absorption, leading to the closure of the lumen on the day of implantation.

Immunohistochemical studies showed that CFTR immunoreactivity was not found in the epithelia, neither in the lumen nor in the glands. The observed downregulation or absence of CFTR in the uterine epithelia ensures that the uterine secretory activity is at a minimum during implantation. Furthermore, the absence of CFTR also enables maximal ENaC activity, since CFTR has been shown to be a negative regulator of ENaC[20].

Taken together, the demonstrated differential expression of CFTR and ENaC during pre-implantation suggests that maximal fluid absorption prior to implantation to ensure the immobilization of the blastocyst necessary for implantation may be a result of: (1) enhanced Na^+ absorption due to upregulated ENaC expression; (2) reduced fluid secretion due to downregulation of CFTR; and (3) maximized ENaC activity, and thus fluid absorption, due to removal of negative regulation by CFTR in its absence.

The differential expression of CFTR and ENaC during the estrous cycle[10], and during implantation provides a mechanism whereby the endometrium can switch from fluid secretion to fluid absorption, depending on physiological needs, i.e., secretion for sperm transport and absorption for implantation. It should be noted that while fluid movement is driven by gradients of electrolytes, which depends largely on ion channels, the flow of fluid across the cell membrane may also require water channels, which are also known to be expressed in the female reproductive tract[23,24]. Interestingly, CFTR is also known to regulate water channels[25]. Therefore, it may require the coordination of ion channels and water channels in regulating the fluid volume in the female reproductive tract, the details of which require further studies.

2.3 Uterine HCO_3^- secretion and sperm capacitation

Apart from regional and cyclic changes in the fluid volume, regional and dynamic changes in the fluid composition have also been noted. Most strikingly, it was observed over 4 decades ago that uterine and oviductal fluids contain 2- to 4-fold higher HCO_3^- content than that in the plasma[26,27] and that its concentration varies along the tract and depends on reproductive events; i.e., increased HCO_3^- during fertilization[28]. However, the cellular mechanisms underlying the formation of the HCO_3^--rich uterine and oviductal fluids remain largely unknown for the past several decades, despite the accumulating evidence indicating the importance of HCO_3^- in sperm function and thus fertilization[29-32].

Using the reconstituted mouse endometrial epithelium model in conjunction with I_{SC} technique together with transporter inhibitors and channel blockers, we have demonstrated concurrent secretion of both Cl^- and HCO_3^- by the mouse endometrial epithelium in response to a number of physiological stimulators, including adrenaline, noradrenaline and PGE_2[8,33]. Further experiments showed that the HCO_3^--mediated current was dependent on basolateral extracellular Na^+ and blocked by basolateral addition of the inhibitor of Na^+-HCO_3^- cotransporter (NBC), H_2DIDS, indicating the involvement of NBC[34]. Another experiment was conducted monitoring intracellular pH (pH_i) recovery from cellular acidification, which was also found to be dependent on both basolateral Na^+ and HCO_3^-, further indicating a basolaterally located NBC responsible for the uptake of HCO_3^- from the blood into endometrial epithelial cells. Later experiments also found the presence of an isoform of Na^+-H^+ exchanger (NHE) in the basolateral membrane[35]. Thus, it appears that basolateral accumulation of HCO_3^- in the endometrial epithelial cells requires two transporters. NBC is responsible for the uptake of HCO_3^- into the cells while NHE may expel H^+ from the cells at the same time to achieve maximal HCO_3^- accumulation (Fig.1).

HCO_3^- extrusion into the uterine lumen has recently been demonstrated to involve an anion exchanger and CFTR, a cAMP-dependent Cl^- channel expressed in the apical membrane of most epithelia, mutations of which are known to result in hallmark defects in Cl^- and HCO_3^- secretion in exocrine glands seen in CF[36,37]. The first evidence

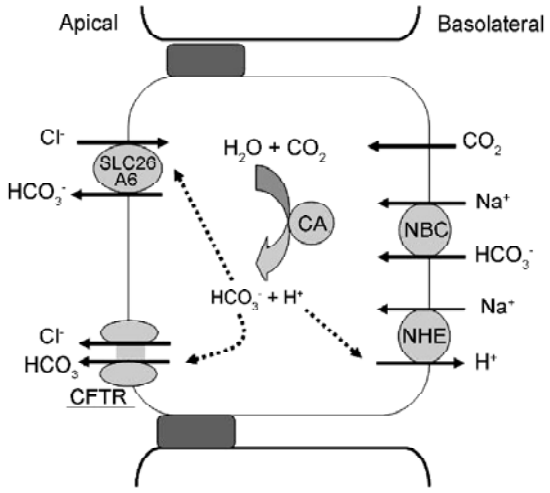

Fig.1. Cellular mechanisms of HCO_3^- secretion in mouse endometrial epithelium. HCO_3^- is secreted into the lumen through apically located ion channel — cystic fibrosis transmembrane conductance regulator (CFTR) or anion exchanger (SLC26A6), and cellular accumulation of HCO_3^- depends on basolaterally situated Na^+/HCO_3^- cotransporter (NBC) and Na^+/H^+ exchanger (NHE). Hydration of CO_2 by the catalyzation of carbonic anhydrase (CA) is also one of the possible sources of HCO_3^-.

supporting their involvement came from pH_i measurements on the reconstituted mouse endometrial epithelium[35]. The rate of pH_i recovery from cellular alkalization was greatly attenuated when extracellular Cl^- was removed, indicating a Cl^--dependent HCO_3^- extrusion mechanism, most likely involving a Cl^-/HCO_3^- exchanger. However, even in the absence of apical Cl^-, which excludes the involvement of the Cl^-/HCO_3^- exchanger, the rate of pH_i recovery from cellular alkalization could still be increased by an adenylate cyclase activator, forskolin, indicating a cAMP-dependent HCO_3^- extrusion pathway. The forskolin induced pH_i recovery could be blocked by glibenclamide (Fig.2), a blocker known to inhibit CFTR, suggesting that CFTR may be responsible for the HCO_3^- secretion. This notion was further supported by the I_{SC} measurements. Forskolin or genistein, a plant-derived compound known to activate CFTR[38], were shown to stimulate a HCO_3^--dependent I_{SC}, which could be completely blocked by CFTR-sensitive DPC or glibenclamide but to a much less extent by CFTR-insensitive DIDS, which is known to inhibit certain types of Cl^-/HCO_3^- exchangers. HCO_3^--dependent I_{SC} could also be suppressed by antisense oligonucleotide against CFTR. Taken together, these results strongly support a direct role of CFTR in mediating uterine HCO_3^- secretion.

As for the oviduct, where sperm capacitation *in vivo* most likely occurs and high HCO_3^- content is also found, no study on its HCO_3^- transporting mechanisms has been reported. However, since CFTR has been found to be expressed and functional in the oviduct[10,14], CFTR may also be responsible for the high HCO_3^- contents found in the oviduct. Studies on porcine oviductal epithelium are currently carried out in the authors' laboratory and preliminary results strongly indicate an oviductal HCO_3^- secretory mechanism similar to that observed for the endometrial epithelium, involving both CFTR and Cl^-/HCO_3^- exchanger, most likely to be salt carrier protein SLC26A6.

Since HCO_3^- is an important factor for sperm capacitation and embryo development[39], the involvement of CFTR in mediating HCO_3^- secretion across uterine and oviductal epithelia suggest that CFTR plays an important role in defining the fluid composition important for these reproductive events, in addition to its role in regulating the fluid volume (see above).

3 Defective or abnormally regulated ion transport under pathological conditions

The function of the epithelial ion channels involved in balancing the secretory and absorptive activities of the reproductive tract epithelia is of paramount importance. This can be highlighted by disorders or diseases with disturbed reproductive events and infertility resulted from defects or abnormal regulation of these ion channels.

3.1 CF

CFTR is a cAMP-regulated Cl^- channel, mutations of which are found to be responsible for disease CF, which affects most of the exocrine glands and tissues including the reproductive tracts[36] (also see review by Quinton in this Issue). Women with CF are known to have reduced fertility rate but the cause remains obscure. The thick cervical mucus resulted from defective electrolyte and fluid secretion due to mutations of CFTR was thought to prevent the penetration of sperm leading to reduced fertility in CF women[40]. However, the demonstrated involvement of CFTR in mediating uterine HCO_3^- secretion and the critical role of HCO_3^- in spermatozoa function, especially sperm capacitation, led us to propose an alternative hypothesis that defective CFTR-mediated HCO_3^- secretion could lead to impaired sperm fertilizing capacity and thus reduced fertility as seen in CF women. This hypothesis was tested by Wang XF *et al*. in a mouse sperm-endometrial epithelial cell co-culture system[39]. Computer assisted sperm analysis (CASA) revealed that sperm motility was greatly enhanced by when sperm was co-cultured with the endometrial epithelia, which have normal HCO_3^- secretion by CFTR,

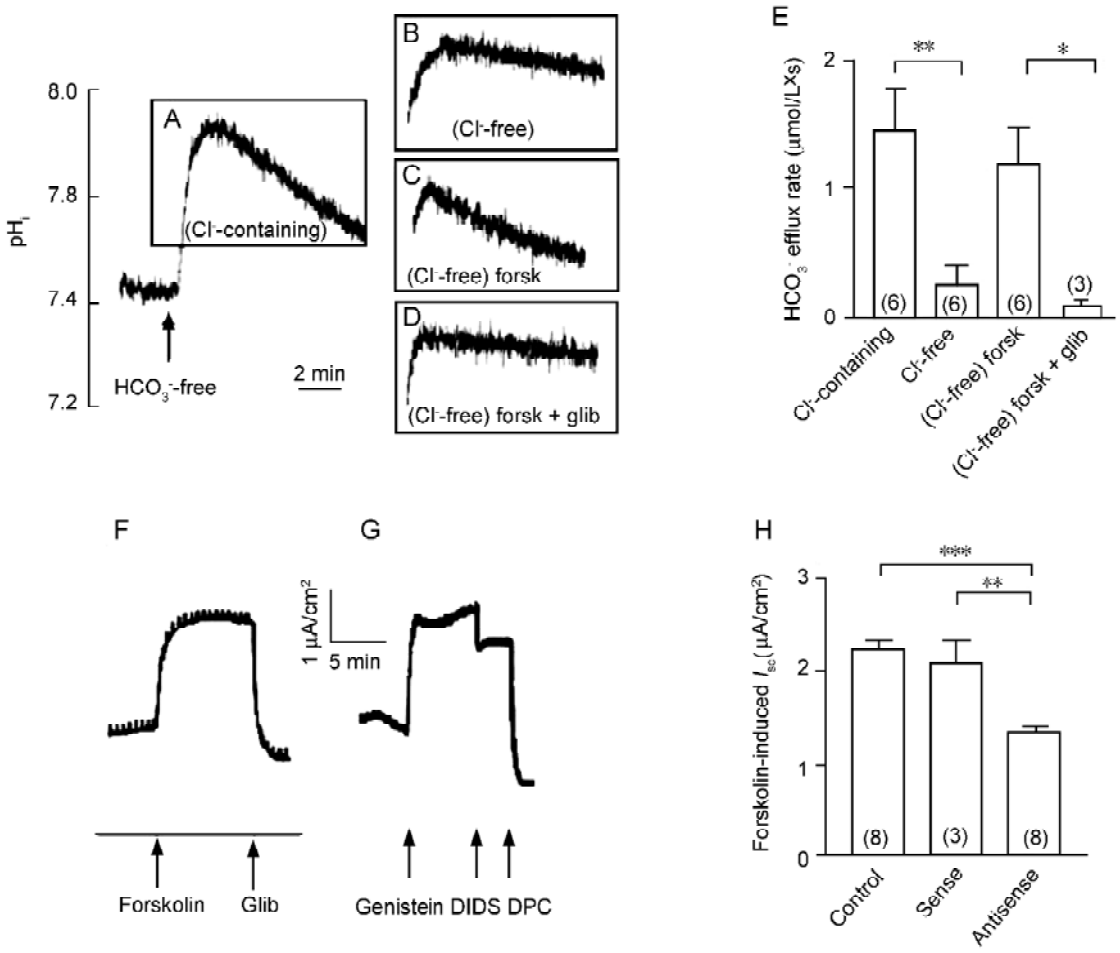

Fig.2. CFTR is the ion channel mainly responsible for HCO_3^- secretion of endometrail epithelium. *A-E*: Intracellular pH (pH_i) of endometrial epithelium recovery after cellular alkalinization induced by removing HCO_3^-/CO_2 from perfusate. *A*: In the presence of Cl^-. *B*: In the absence of Cl^-. *C*: In the absence of Cl^- and stimulated with forskolin (forsk, 10 μmol/L. *D*: In the absence of Cl^- and treated with forskolin and glibenclamide (glib, 200 μmol/L). *E*: Summary of HCO_3^- efflux rates under different conditions. $^*P<0.05$, $^{**}P<0.01$. *F*, *G*: HCO_3^--dependent short-circuit current (I_{SC}) across endometrial epithelia stimulated by cAMP-evoking agents and blocked by CFTR inhibitors (glibenclamide, 1 mmol/L; DPC, 1 mmol/L). *H*: Summary of effect of CFTR antisense on forskolin-induced I_{SC}. $^{**}P<0.01$, $^{***}P<0.001$. (Originally published by Wang *et al*. in Nature Cell Biol 2003; 5(10): 902-906[39]).

as compared to that incubated with HCO_3^--free or endometrium cell-free medium. In addition, the percentage of capacitated sperm was significantly attenuated when CFTR expression in the endometrial epithelial cells was suppressed with antisense against CFTR, as compared to the sense-treated controls. *In vitro* fertilization (IVF) assays on zona-intact mouse eggs further demonstrated that the number of 2-cell embryos as an indication of fertilization obtained with sperm capacitated in conditioned medium from CFTR antisense-treated endometrial cells was significantly reduced as compared to that obtained from sense-treated controls. Taken together, these results suggested that CFTR-mediated uterine HCO_3^- secretion is important for sperm capacitation and that impaired HCO_3^- secretion caused by defective CFTR results in reduced sperm fertilizing capacity.

These results have provided an alternative explanation, other than the thick cervical mucus, for reduced fertility seen in CF women. In fact, clinical studies on CF women undergoing assisted reproduction showed that pregnancy could not be achieved in some patients even after repeated sperm insemination, a procedure to overcome the thick cervical mucus long thought to be the defect in CF, but succeed only with IVF[41]. These clinical cases suggest defects beyond the cervix in CF women leading to infertility, which is consistent with our findings. It appears that impaired HCO_3^- secretion along the female tract due to defects in either transport mechanisms (apical or basolateral)

or its regulation (neural or hormonal) could also be the cause of some unexplained female infertility since other reproductive events, such as embryo development, are also known to be dependent on HCO_3^-[42]. Interestingly, our recent studies have also demonstrated the expression of CFTR in sperm and its involvement in mediating HCO_3^- entry into sperm, which is necessary for capacitation[43], indicating the far-reaching effect of CFTR in both male and female reproduction.

3.2 Ovarian hyperstimulation syndrome (OHSS)

OHSS is one of the most life-threatening and potentially fatal complications of assisted reproduction treatments, arising from excessive stimulation of the ovaries by exogenous gonadotropins administrated during IVF procedures, which is characterized by massive fluid shift and accumulation in the peritoneal cavity and other organs, including the lungs and the reproductive tract. The pathogenesis of OHSS remains obscure, and no definitive treatments are currently available. Rapid passage of fluid into luminal spaces, as seen in OHSS, may be a consequence of abnormal ion transport across the epithelia. It has also been well established that estrogen levels are highly elevated during ovarian hyperstimulation, with excessively high levels observed in OHSS[44-46]. We hypothesized that abnormally upregulated CFTR expression and function by the excessively high levels of estrogen may be the cause of OHSS. In an OHSS rat model, OHSS symptoms as well as upregulated CFTR expression and enhanced CFTR channel activity were observed, which could be mimicked by administration of estrogen, but not progesterone, alone in ovariectomized rats. Administration of progesterone that suppresses CFTR expression or antiserum against CFTR to OHSS animals resulted in alleviation of the symptoms. Furthermore, ovarian hyperstimulation did not induce detectable OHSS symptoms in CFTR mutant mice, confirming involvement of CFTR in the pathogenesis of OHSS. Here we have de-monstrated a pathological condition caused by abnormally upregulated CFTR with increased channel activity leading to excessive fluid shift and accumulation in the peritoneal cavity and in different organs, a condition that is in great contrast to CF, a disease with hallmark defect in electrolyte and fluid transport in most exocrine glands due to defective CFTR[36]. Interestingly, both OHSS and CF could be life-threatening. Taken together, these data further confirm a critical role for CFTR in regulating body electrolyte and fluid secretion, an abnormality of which, in either expression or function, could result in lethal conditions, as seen in both CF and OHSS.

3.3 Hydrosalpinx (HSP)

HSP is characterized by abnormal fluid accumulation in the tubes, with unknown etiology but generally accepted to be due to bacterial infections, which accounts for about 30% of tubal factor infertility but has received little attention. The apparent fluid disturbance in HSP led us again to hypothesize that CFTR might be involved in the pathogenesis since CFTR is expressed in the oviduct[10] and a functional cAMP-activated Cl^- conductance is present in normal but not CFTR mutant mice[14,47]. We tested this hypothesis by examining epithelial pathology and CFTR expression in the Fallopian tubes of infertile HSP patients seeking assisted reproduction treatments. Masson's trichrome staining showed areas of epithelial transformation, focally attenuated and pseudostratified. Immunostaining showed enhanced CFTR immunoreactivity in the focally attenuated and pseudostratified areas of HSP epithelium. Significantly increased CFTR mRNA expression was also observed in HSP compared with in normal Fallopian tubes. Thus, abnormally upregulated CFTR may be one of the underlying mechanisms for abnormal fluid secretion and accumulation in the HSP lumen, but the cause for abnormal upregulation of CFTR remains unknown. An understanding of the mechanisms underlying HSP formation following pelvic inflammatory disease appears to be essential in elucidating the causes for reduced implantation in HSP patients and providing more rational treatments.

3.4 Infection

Although the exact reason for the increased expression of CFTR observed in HSP remains elusive, one possible cause may be due to infection. It has been reported that bacterial infections upregulate CFTR expression[48]. Cytokines produced during infection[49], such as interleukin 1β (IL-1β), are potent modulators of CFTR expression[50]. During infection, bacterial binding may stimulate receptor-coupled signalling, leading to protein tyrosine phosphorylation, and finally increased CFTR expression. It has been reported that chlamydial infection alters the transcription of host cell genes including those for cell differentiation, transcription factors and inhibition of apoptosis[51]. Chlamydial chronic infection, a currently considered leading cause of HSP, may have contributed, at least in part, to the increased CFTR expression. It has also been demonstrated that *C. trachomatis* infection results in increased tyrosine phosphorylation of several host proteins including those involved in signal transduction pathways[51-53]. In fact, our preliminary data showed that *C. trachomatis* inoculated into healthy Sprague-Dawley rat uteri induced uterine infection, massive uterine fluid accumulation (as seen in HSP) and increased CFTR mRNA expression[54], supporting the notion that infection

may lead to upregulation of CFTR with concomitant changes in ion flux across the Fallopian tubal epithelium accompanied with increased fluid accumulation resulting in HSP.

Accumulation of HSP fluid in the Fallopian tubes and its regurgitation into the uterine cavity may be a contributing factor for infertility observed in HSP patients, with impaired implantation or endometrium receptivity of transferred embryos during IVF[55,56]. Interestingly, improved IVF outcome has been observed in HSP patients pretreated with antibiotics[57,58], which may be due to the return of CFTR expression back to the normal level when infection had subsided, leading to reduced HSP fluid reflux into the uterine cavity. Salpingectomy prior to IVF has been reported to be beneficial[59-61], and currently, salpingectomy for large HSP prior to IVF is an accepted practice. However, most patients are reluctant to consent to this procedure. If treatment with CFTR specific inhibitors (i.e., local administration) in conjunction with antibiotics improves IVF and embryo transfer outcome in a clinical trial, it will certainly be a more attractive option to most patients than salpingectomy, and therefore this option as the potential adjunct treatment for HSP should be evaluated.

4 Conclusions and unanswered questions

Recent studies have begun to unravel the molecular mechanisms regulating the fluid microenvironment of the female reproductive tract, on which various reproductive events critically depend. It is now clear that epithelial ion channels, particularly CFTR and ENaC, and their regulation by ovarian hormones are responsible for the cyclic changes in fluid volume and composition, and that defects in these ion channels and their regulation may lead to disturbance of the fluid microenvironment resulting in various pathological conditions and infertility. However, there are many unanswered questions. For example, while both CFTR and ENaC have been found to be expressed along the female genital tract and the expression of CFTR seems to inhibit ENaC function, are these channels actually colocalized? Do they interact with each other at protein level? If yes, then how? The answers to these questions would be important for the understanding of how the secretory and absorptive activities of the genital tract epithelia may be governed during the cycle, and how the reproductive events, such as sperm passage and implantation, may be facilitated and even timed by the fluid microenvironment. Along this line it comes another question as to whether water channels are involved in regulating the fluid microenvironment by interacting with CFTR. The abundance of water channels expressed along the female genital tract certainly indicates their involvement but how they contribute to the regulation of fluid volume requires detailed studies. Another important aspect is the host defense mechanism against bacterial infections in the female reproductive tract. What changes in the fluid microenvironment would be brought about upon bacterial infections? What are the physiological or pathophysiological consequences to these changes? We have just begun to look into this and our preliminary data suggest that HCO_3^- may play an important role in the host defense. Together with its demonstrated role in sperm capacitation as well as its potential role in a number of other reproductive processes, HCO_3^- appears to be a versatile bioactive molecule essential to various reproductive functions. However, the details regarding HCO_3^- transport across different regions and the mechanisms determining its rate of transport under various conditions, such as upon bacterial infection, remain largely unknown. Considering the involvement of CFTR in uterine/oviductal HCO_3^- secretion, and its observed upregulation upon bacterial infection, it is almost certain that CFTR plays an important role in the host defense against bacterial infection in the female reproductive tract as well. However, is CFTR working alone or interacting with other transporters, such as the anion exchanger, in regulating HCO_3^- secretion in the female genital tract? (for interaction between CFTR and anion exchangers see review by Ishiguro *et al*. in this Issue) Further studies along the questions raised above will enable our better understanding of the molecular mechanisms regulating the fluid microenvironment that determines the outcome of fertility, and even life and death, as in the case of OHSS.

* * *

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