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human reproduction update

Defensins: defenders of human reproductive health

Yu-Jia Zhai[1,2,†], Ying Feng[3,†], Xue Ma[4,*], and Fang Ma[1,2,*]

[1]Center for Translational Medicine, Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, P.R. China [2]Department of Obstetrics and Gynecology, West China Second Hospital, Sichuan University, Chengdu, Sichuan, China [3]Department of Histology, Embryology, and Neurobiology, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University [4]Department of Pediatric Urology, West China Hospital, Sichuan University, Chengdu, Sichuan, China

*Correspondence address. Center for Translational Medicine, Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, West China Second University Hospital, Sichuan University, Chengdu, Sichuan 610041, China. E-mail: mafangmed@126.com https://orcid.org/0000-0002-7781-821X (F.M.); Department of Pediatric Urology, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China. E-mail: medmaxue@163.com https://orcid.org/0000-0002-7650-6214 (X.M.)

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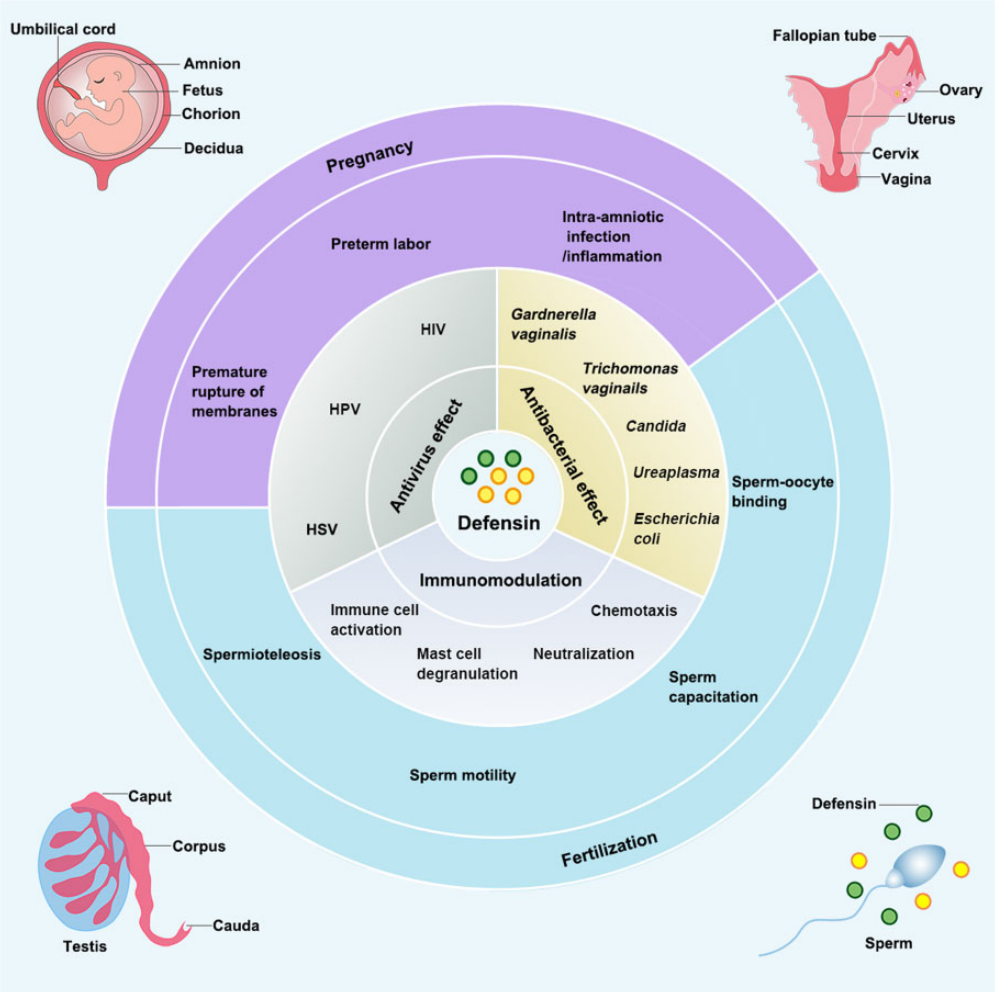
TABLE OF CONTENTS

[†]These authors contributed equally to this work.

GRAPHICAL ABSTRACT



The dual roles of defensins in the human reproductive system: host defence and protection of fertility.

BACKGROUND: Reproductive tract infection is an important factor leading to male and female infertility. Among female infertility factors, microbial and viral infections are the main factors affecting female reproductive health and causing tubal infertility, ectopic tubal pregnancy and premature delivery. Among male infertility factors, 13–15% of male infertility is related to infection. Defensins are cationic antibacterial and antiviral peptides, classified into α-defensins, β-defensins and θ-defensins. Humans only have α-defensins and β-defensins. Apart from their direct antimicrobial functions, defensins have an immunomodulatory function and are involved in many physiological processes. Studies have shown that defensins are widely distributed in the female reproductive tract (FRT) and male reproductive tract (MRT), playing a dual role of host defence and fertility protection. However, to our knowledge, the distribution, regulation and function of defensins in the reproductive tract and their relation to reproduction have not been reviewed.

OBJECTIVE AND RATIONALE: This review summarizes the expression, distribution and regulation of defensins in the reproductive tracts to reveal the updated research on the dual role of defensins in host defence and the protection of fertility.

SEARCH METHODS: A systematic search was conducted in PubMed using the related keywords through April 2022. Related data from original researches and reviews were integrated to comprehensively review the current findings and understanding of defensins in the human reproductive system. Meanwhile, female and male transcriptome data in the GEO database were screened to analyze defensins in the human reproductive tracts.

OUTCOMES: Two transcriptome databases from the GEO database (GSE7307 and GSE150852) combined with existing researches reveal the expression levels and role of the defensins in the reproductive tracts. In the FRT, a high expression level of α-defensin is found,

and the expression levels of defensins in the vulva and vagina are higher than those in other organs. The expression of defensins in the endometrium varies with menstrual cycle stages and with microbial invasion. Defensins also participate in the local immune response to regulate the risk of spontaneous preterm birth. In the MRT, a high expression level of β-defensins is also found. It is mainly highly expressed in the epididymal caput and corpus, indicating that defensins play an important role in sperm maturation. The expression of defensins in the MRT varies with androgen levels, age and the status of microbial invasion. They protect the male reproductive system from bacterial infections by neutralizing lipopolysaccharide and downregulating pro-inflammatory cytokines. In addition, animal and clinical studies have shown that defensins play an important role in sperm maturation, motility and fertilization.

WIDER IMPLICATIONS: As a broad-spectrum antimicrobial peptide without drug resistance, defensin has great potential for developing new natural antimicrobial treatments for reproductive tract infections. However, increasing evidence has shown that defensins can not only inhibit microbial invasion but can also promote the invasion and adhesion of some microorganisms in certain biological environments, such as human immunodeficiency virus. Therefore, the safety of defensins as reproductive tract anti-infective drugs needs more in-depth research. In addition, the modulatory role of defensins in fertility requires more in-depth research since the current conclusions are based on small-size samples. At present, scientists have made many attempts at the clinical transformation of defensins. However, defensins have problems such as poor stability, low bioavailability and difficulties in their synthesis. Therefore, the production of safe, effective and low-cost drugs remains a challenge.

Key words: defensin / reproductive tract / fertility / infertility / maternal–fetal interface / sperm / premature delivery / antibacterial / antivirus / immunomodulation

Introduction

The mucosal innate immunity provides rapid and nonselective protection for the mucosa of the digestive, respiratory and urogenital system via the uppermost mucus lining, a cellular junctional complex-linked epithelial barrier, along with antimicrobial peptides and cytokines secreted by epithelial and innate immune cells (Wira *et al.*, 2005). Among various antimicrobial peptides expressed in the human body, defensins are one of the most abundantly expressed classes. They are a class of cationic peptides expressed predominantly by neutrophils and epithelial cells and they act as an essential component in the mucosal innate immune response against infectious pathogens (Lehrer and Lu, 2012). Many comprehensive reviews have discussed the complex mechanism of the antimicrobial activity of defensins and their roles in the mucosal immunity of the digestive and respiratory systems (Schutte and Mccray, 2002; Ganz, 2003; Klotman and Chang, 2006; Ouellette, 2011; Xu and Lu, 2020).

Like the digestive and respiratory mucosa, reproductive tract mucosa is constantly exposed to endogenous and exogenous microbial invasion pressure. Different sources of pathogen invasion can lead to sexually transmitted diseases (STDs), endogenous infections or iatrogenic infections, which are classified as reproductive tract infections. They affect not only the patients' health but also cause adverse reproductive outcomes, such as infertility, premature delivery and miscarriage (Brunham and Paavonen, 2020; Sharma *et al.*, 2022). In the reproductive system, defensins are widely expressed, and their expression levels are modulated by hormone levels, age and microbial invasion (Quayle *et al.*, 1998; Fleming *et al.*, 2003; Cao *et al.*, 2010; Yu *et al.*, 2013). They are an important part of the innate immune defence system of the reproductive tract mucosa, which can protect the reproductive tract from microorganisms. In addition, defensins expressed in the reproductive systems are also involved in various reproductive events, such as sperm maturation, sperm transportation, sperm–oocyte binding, implantation, pregnancy process, fetal development and childbirth (Tollner *et al.*, 2011, 2012; Burris *et al.*, 2020). This review summarizes the expression, distribution and regulation of defensins in the female reproductive tract (FRT) and male reproductive tract (MRT) and shows the progress of research on the dual roles of defensins in host defence and the protection of reproductive tract fertility.

Methods

A comprehensive search of the scientific literature on PubMed was conducted through to April 2022 using the following keywords and phrases in combination with each other; 'defensins', 'defensins general structure', 'defensins classification and expression', 'defensins and sterility', 'defensins and pregnancy', 'defensins and fertilization', 'defensins and preterm birth', 'defensins and reproductive tract', 'defensins and sperm', 'defensins and epididymis', 'defensins and testis', 'defensins and prostate', 'defensins and estrogen', 'defensins and androgen', 'defensins and vaginal microbiota', 'defensins and female microbiota', 'defensins and male microbiota', 'defensins and microbial infections', 'defensins and viral infections', 'defensins and sexually transmitted diseases', 'defensins and HIV', 'defensins and HPV', 'defensins and bacterial vaginosis', 'defensins and *Candida*', 'defensins and *Mycoplasma*', 'defensins and *Neisseria gonorrhoeae*', 'defensins and *G. vaginalis*', 'defensins and *Escherichia coli*', 'defensins and *Ureaplasma*', 'defensins and *Trichomonas vaginalis*'. Related data and conclusions from original research and reviews were integrated to comprehensively review the current findings and understanding of defensins in the human reproductive system. In certain parts where human research was limited, data from animal studies were used for supplementation.

Meanwhile, transcriptome data were searched in the GEO database using the keywords: 'female reproductive tract' and 'male reproductive tract'. GeneChip array data GSE7307 and high-throughput sequencing data GSE150852 were screened to analyze defensins in the human reproductive tracts.

Defensins

Classification of defensins

Defensins are a class of cationic and Cys-rich peptides with 18–45 amino acids (2–5 kDa) with conserved three-dimensional structures: prominent β-sheets stabilized by three disulfide bonds (Pazgier *et al.*, 2006; Lehrer and Lu, 2012; Shafee *et al.*, 2017). According to the position of the disulfide bond, they are grouped into α-defensins, β-defensins and θ-defensins (Selsted *et al.*, 1993; Tang *et al.*, 1999; Lehrer, 2004; Lehrer *et al.*, 2012). Alpha-defensins and β-defensins are conserved in humans and other animals, while θ-defensins are cyclic peptides found only in the white blood cells of non-human primates (Cole *et al.*, 2002; Lehrer *et al.*, 2012).

α-defensins

Six α-defensins have been identified in humans so far; based on their expression pattern and genetic organization, they are subdivided into myeloid and enteric peptides (Selsted and Ouellette, 2005). Lehrer's group first identified three abundantly expressed human myeloid α-defensins in the azurophilic granules of neutrophils and named these peptides human neutrophil peptides 1, 2 and 3 (HNP1, HNP2, HNP3) in 1985 (Ganz *et al.*, 1985). Although encoded by different genes (*DEFA1* and *DEFA3*), HNP1 and HNP3 only differ at the first residue at the N-terminus, and HNP2 is the proteolytic product of either HNP1 or HNP3 (Sparkes *et al.*, 1989; Linzmeier *et al.*, 1993; Mars *et al.*, 1995; Holly *et al.*, 2017). HNP4, the last identified myeloid α-defensin, exhibits the same cysteine framework and structural similarity to HNP1–3 (Gabay *et al.*, 1989; Wilde *et al.*, 1989). However, the amino acid sequence of HNP4 is significantly different from that of HNP1–3 and this results in a notable functional difference between them (Hu *et al.*, 2019). HNP1–4 are synthesized in the promyelocytic bone marrow precursors, and up to 30–50% of the proteins in the azurophilic granules are HNP1–3, but there is a hundred times lower amount of HNP4 (Gabay *et al.*, 1989). They can also be found in monocytes/macrophages, natural killer cells, T cells, B cells and immature dendritic cells (DCs) (Selsted and Ouellette, 2005; Pazgier *et al.*, 2007). The other two enteric α-defensins, human defensin 5 and 6 (HD5 and HD6), share almost the same three-dimensional structure as the myeloid α-defensins but have distinct amino acid compositions. The two peptides are mainly expressed in Paneth cells at the bottom of the intestinal glands (or the crypt of Lieberkühn) (Jones and Bevins, 1992; 1993). Moreover, HD5 is also expressed in the epithelial cells of the male and female urogenital tracts (Quayle *et al.*, 1998; Com *et al.*, 2003; Ouellette, 2011; Spencer *et al.*, 2012). A more detailed structure and sequence information of human α-defensins can be found in the reviews written by Chang, Lu and Lehrer (Klotman and Chang, 2006; Pazgier *et al.*, 2007; Lehrer and Lu, 2012).

β-defensins

From the first human β-defensin, HBD1, identified in the plasma of a kidney disease patient in 1995, 34 β-defensin genes (including three pseudogenes: *DEFB109A*, *DEFB117* and *DEFB122*) are now listed in the NCBI gene database (Bensch *et al.*, 1995). However, not all have been studied at the protein level (Table I) and only four have been studied in detail (HBD1–4). The amino acid composition of β-defensins is significantly different from that of the α-defensins, resulting in a three-stranded β-sheet structure that can be further divided into hydrophobic and charged domains (Pazgier *et al.*, 2006). Chromosome 8p21-p23 region was the first identified gene cluster to encode the β-defensin proteins HBD1–3 (corresponding genes: *DEFB1*, *DEFB4* and *DEFB103*), and this region also contains all α-defensin protein genes. This finding indicates that there is a common ancestral defensin gene. Subsequently, three other *DEFB* gene clusters were found on chromosomes 6p12, 20q11.1 and 20p13 (Bevins *et al.*, 1996; Liu *et al.*, 1997; Schutte *et al.*, 2002). Compared with the restricted expression of α-defensins, the expression of β-defensins is more widely distributed in various tissues in the human body. The major expression sites include skin keratinocyte and epithelial cells of the reproductive, urinary, digestive and respiratory tracts (O'Neil, 2003; Pazgier *et al.*, 2006).

θ-defensins

Naturally synthesized θ-defensins are only found in certain non-human primates, such as rhesus macaques (Tang *et al.*, 1999) and olive baboons (Garcia *et al.*, 2008). Different from the structure of α-defensin and β-defensin, the connecting positions of disulfide bonds in the molecular chain of θ-defensin are Cys1-Cys4, Cys2-Cys5 and Cys3-Cys6, forming a ring structure (Lehrer *et al.*, 2012). Human bone marrow contains mRNA very similar to the rhesus θ-defensin precursor mRNA, while a stop codon exists in its signal sequence, resulting in the failure to produce human endogenous θ-defensin peptides (Lehrer *et al.*, 2012). However, the pseudogene sequences provided the sequence foundation for *in vitro* synthesis of the functional peptides. Lehrer and colleagues hence reconstructed humanized θ-defensins based on these sequences via cloning, peptide synthesis and molecular archaeology and named them 'retrocyclins' (Cole *et al.*, 2002). The unique cyclic structure allows θ-defensins to show more resistance to high salts and proteases, which are advantages in antimicrobial activities.

General function of defensins

Firstly discovered as a class of antibacterial peptides, human defensins also exhibit antiviral, antifungal and immunomodulatory effects in inflammation, development and cancer (Lai and Gallo, 2009; Semple and Dorin, 2012; Holly *et al.*, 2017; Ordonez *et al.*, 2017; Jin and Weinberg, 2019) (Fig. 1). Among the various functions, the antibacterial function of defensins is the most well-studied. Based on their positively charged character, defensins can kill bacteria or inhibit bacterial growth through different mechanisms, including directly punching holes in the negatively charged bacterial membrane (Lehrer and Lu, 2012) and inhibiting bacterial cell wall synthesis via interaction with the cell wall precursor lipid II (De Leeuw *et al.*, 2010). They can also neutralize bacterial secreted toxins to reduce bacterial infection (Kim *et al.*, 2005). The antibacterial mechanism of defensins mainly includes three steps: (i) the positively charged defensins bind with the negatively charged bacterial membrane by electrostatic attraction; (ii) defensin molecules or their polymers interact with phospholipid heads and water molecules on the bacterial plasma membrane to form a channel, which significantly increases the permeability of the biological membrane; and (iii) bacterial inner cell content leakage occurs (Lehrer *et al.*, 1989; Zasloff, 2002; Selsted and Ouellette, 2005; Shafee *et al.*, 2017). The detailed mechanisms of bacterial killing by human α-defensins and β-defensins can be different because α-defensins are more hydrophobic (Lehrer and Lu, 2012). Different β-defensins show distinct preferences for various bacteria: HBD1 and HBD2 are more active

Table I The expression of defensins in the human reproductive tracts.

Gene symbols	Female reproductive tract			Male reproductive tract		
	Expression site	Detected molecule	Reference	Expression site	Detected molecule	Reference
DEFA1–3 (HNP1–3)	Vaginal fluid	Protein	(Svinarich *et al.*, 1997; Hein *et al.*, 2002; Akinbi *et al.*, 2004; Das *et al.*, 2007, 2008; Lucovnik *et al.*, 2011)	Testis	mRNA	(Com *et al.*, 2003)
	Cervical mucus	Protein				
	Endometrium	Protein				
	Follicular fluid	Protein				
	Amniotic fluid	Protein		Spermatozoa	mRNA	
	Amnion	mRNA				
	Chorion	mRNA				
	Decidua	mRNA				
DEFA4 (HNP4)	Not reported			Not reported		
DEFA5 (HD5)	Vagina	Protein and mRNA	(Quayle *et al.*, 1998)	Testis	mRNA	(Com *et al.*, 2003)
	Cervix	Protein and mRNA				
	Endometrium	Protein and mRNA				
	Fallopian tube	Protein and mRNA		Spermatozoa	mRNA	
	Cervicovaginal lavages	Protein				
DEFA6 (HD6)	Vagina	mRNA	(Fichorova *et al.*, 1997; Klotman *et al.*, 2008)	Testis	mRNA	(Com *et al.*, 2003)
	Ectocervix	mRNA				
	Endocervix	mRNA				

Gene symbols	Female reproductive tract			Male reproductive tract		
	Expression site	Detected molecule	Reference	Expression site	Detected molecule	Reference
DEFB1 (HBD1)	Amnion epithelium	Protein	(Zhao *et al.*, 1996; Hein *et al.*, 2002; King *et al.*, 2002, 2007a,b; Erhart *et al.*, 2011; Noda-Nicolau *et al.*, 2017)	Semen	Protein	(Zhao *et al.*, 1996; Com *et al.*, 2003; Zupin *et al.*, 2019a)
	Chorion trophoblast	Protein				
	Decidua	Protein		Testis	Protein and mRNA	
	Placental syncytiotrophoblast	Protein				
	Vagina	Protein and mRNA		Spermatozoa	Protein and mRNA	
	Cervicovaginal fluid	Protein				
DEFB1 (HBD1)	Amniotic fluid	Protein	(King *et al.*, 2002; Varrey *et al.*, 2018)	Prostate glands	Protein and mRNA	(Hong *et al.*, 2017)
	Endometrium	mRNA				
	Placenta	mRNA				
DEFB2 (HBD2)	Amnion epithelium	Protein	(King *et al.*, 2002; Soto *et al.*, 2007; King *et al.*, 2007a,b; Erhart *et al.*, 2011; Noda-Nicolau *et al.*, 2017)	Not reported		
	Chorion trophoblast	Protein				
	Decidua	Protein				
	Placental syncytiotrophoblast	Protein				
	Amniotic fluid	Protein				
	Vagina	Protein and mRNA				

(continued)

Table I Continued

Gene symbols	Female reproductive tract			Male reproductive tract		
	Expression site	**Detected molecule**	**Reference**	**Expression site**	**Detected molecule**	**Reference**
	Cervicovaginal fluid	Protein				
	Endometrium	mRNA				
Genes (and proteins)	**Female reproductive tract**			**Male reproductive tract**		
	Expression site	**Detected molecule**	**Reference**	**Expression site**	**Detected molecule**	**Reference**
DEFB3 (HBD3,DEFB103A)	Amnion epithelium and mesenchyme	Protein	(King *et al.*, 2002, 2003a,b, 2007a,b; Erhart *et al.*, 2011; Noda-Nicolau *et al.*, 2017; Para *et al.*, 2020)	Not reported		
	Amniotic fluid	Protein				
	Chorion trophoblast	Protein				
	Decidua	Protein				
	Placental syncytiotrophoblast	Protein				
	Vagina	Protein and mRNA				
	Cervicovaginal fluid	Protein				
	Endometrium	mRNA				
DEFB4 (HBD4,DEFB104A)	Chorioamniotic membranes	mRNA	(King *et al.*, 2003a,b; Polettini *et al.*, 2011)	Epididymis	mRNA	(Lehrer and Ganz, 2002; Yamaguchi *et al.*, 2002)
	Endometrium	mRNA		Testis	mRNA	
DEFB5 (DEFB105A, DEFB105B)	Not reported			Testis	mRNA	(Yamaguchi *et al.*, 2002; Semple *et al.*, 2003)
				Epididymis	mRNA	
DEFB106 (DEFB106A, DEFB-6)	Not reported			Testis	mRNA	(Yamaguchi *et al.*, 2002; Semple *et al.*, 2003; Xin *et al.*, 2014)
				Epididymis	Protein and mRNA	
DEFB107 (DEFB107A)	Not reported			Testis	mRNA	(Semple *et al.*, 2003)
Gene symbols	**Female reproductive tract**			**Male reproductive tract**		
	Expression site	**Detected molecule**	**Reference**	**Expression site**	**Detected molecule**	**Reference**
DEFB108 (DEFB108A/B/C)	Not reported			Testis	mRNA	(Semple *et al.*, 2003)
DEFB109 (DEFB109B/C)	Not reported			Testis	mRNA	(Schutte *et al.*, 2002; Patil *et al.*, 2005)
				Prostate	mRNA	
DEFB114	Not reported			Epididymis	Protein and mRNA	(Yu *et al.*, 2013)
DEFB118	Not reported			Sperm	Protein	(Yenugu *et al.*, 2004)
DEFB119 (DEFB120)	Not reported			Testis	mRNA	(Radhakrishnan *et al.*, 2005)
				Epididymis	mRNA	
DEFB121	Not reported			Testis	mRNA	(Radhakrishnan *et al.*, 2005)
				Epididymis	mRNA	

(continued)

Table I Continued

Gene symbols	Female reproductive tract			Male reproductive tract		
	Expression site	Detected molecule	Reference	Expression site	Detected molecule	Reference
DEFB123	Not reported			Testis	mRNA	(Radhakrishnan *et al.*, 2005)
				Epididymis	mRNA	
DEFB124	Not reported			Prostate epithelia	Protein and mRNA	(Kim *et al.*, 2014)
				Epididymis	mRNA	
DEFB125–128 (DEFB25–28)	Not reported			Testis	mRNA	(Rodríguez-Jiménez *et al.*, 2003)
				Epididymis	mRNA	
DEFB129	Not reported			Testis	mRNA	(Rodríguez-Jiménez *et al.*, 2003; Dubé *et al.*, 2008)
	Not reported			Epididymis	Protein and mRNA	
DEFB131	Not reported			Prostate epithelia	Protein and mRNA	(Kim *et al.*, 2015)
DEFB132	Not reported			Epididymis	Protein	(Lin *et al.*, 2008)
	Not reported			Sperm	Protein	

Gene symbols	β-defensin mRNAs detected in the transcriptome analysis but not reported in the published articles on reproductive tracts		
	Expression site	Detected molecule	Reference
DEFB110	Not reported		(Schutte *et al.*, 2002)
DEFB111-112	Gingival tissues and keratinocytes	mRNA	(Schutte *et al.*, 2002; Premratanachai *et al.*, 2004)
DEFB113	Not reported		(Schutte *et al.*, 2002)
DEFB115-117	Not reported		(Schutte *et al.*, 2002)
DEFB122	Not reported		(Schutte *et al.*, 2002)
DEFB130	Not reported		(Schutte *et al.*, 2002)
DEFB133	Not reported		(Schutte *et al.*, 2002)

against Gram-negative bacteria (Harder *et al.*, 1997), while HBD3 is active against both Gram-positive and -negative strains (Harder *et al.*, 2001).

Defensins have a direct inhibitory effect on viral infections. The degree of inhibition of viruses depends on the concentration of defensins and the tightness of intramolecular disulfide bonds. Its antiviral efficacy is also impacted by time, pH, ionic strength and temperature. Defensins have strong antiviral activity under neutral and low ionic strength conditions (Holly *et al.*, 2017). The α-defensins and β-defensins are sensitive to enveloped viruses. Furthermore, the antiviral activity is executed by inhibiting the interaction between viral glycoproteins and the receptors on the plasma membrane of target cells, which leads to the interruption of the fusion between viral particles and the host cell. Alternatively, they can be absorbed by the negatively charged glycoprotein on the surface of the enveloped virus, therefore perforating the virus particles and forming a breach on their surface, consequently leading to a leak of viral contents and death of the virus (Holly *et al.*, 2017). For non-enveloped viruses, α-defensins prevent infection through direct interaction with the viral capsid, preventing uncoating and leading to internalized viruses that mislead lysosomal degradation. Unlike enveloped viruses, non-enveloped viruses have not been reported to be significantly affected by β-defensins (Wiens and Smith, 2017). In addition, HNP1–3 also execute their antiviral activity by inhibiting the protein kinase C (PKC) signaling pathway involved in virus fusion, transcription, integration and assembly (Yu *et al.*, 1995; Chang *et al.*, 2005).

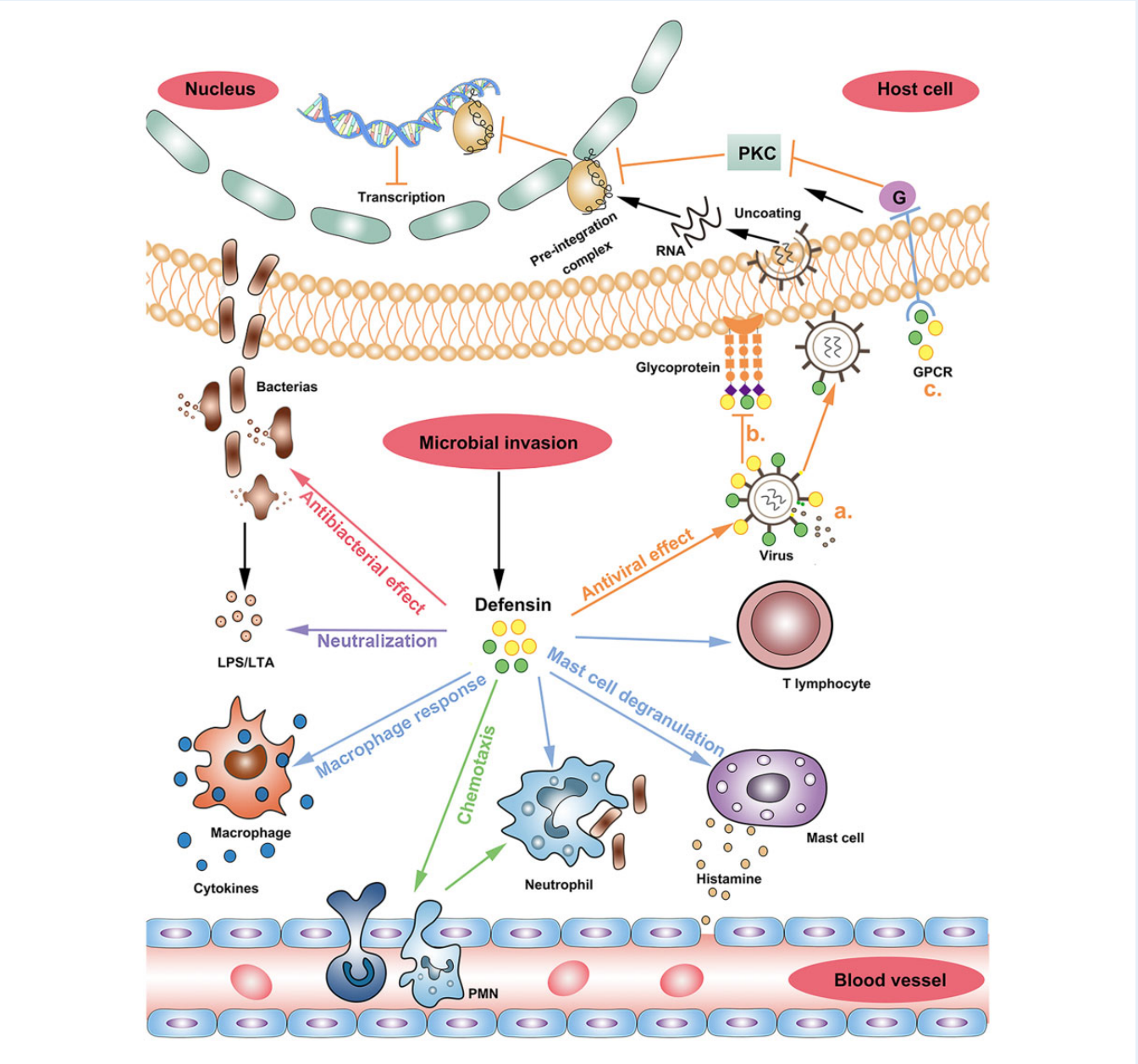


Figure 1. Host defence functions of defensins. Different arrow colors represent different functions of defensins. Red arrow: antibacterial effect. Purple arrow: neutralization effect. Blue arrow: immune cell activation (including activating the phagocytosis of macrophages and neutrophils, promoting histamine release from mast cells, increasing vascular permeability and recruiting T cells to regulate specific immunity). Green arrow: chemotaxis. (Defensins stimulate the chemotaxis of peripheral blood monocyte and neutrophils to reach the infection site quickly.) Orange arrow: antiviral effect. a. The defensins bind to glycoprotein receptors of the virus and the cell surface, restricting the virus from entering the cell; b. The defensin pierces the virus surface, causing its contents to leak out and death; c. When a virus infects host cells, defensins inhibit the transcription of the virus by inhibiting the PKA signaling pathway. LPS/LTA, lipopolysaccharide/lipoteichoic acid; PKC, protein kinase C; GPCR, G-protein coupled receptor; PMN, polymorphonuclear neutrophil.

Defensins also show immunomodulatory effects in both innate and adaptive immune responses. They promote the release of chemokines and activate or mobilize macrophages and mast cells to reach tissues infected by pathogens (Chertov *et al.*, 1996). In addition, defensins can induce the maturation of DCs, mobilize DCs and enhance their antigen uptake ability (Yang *et al.*, 2007). Studies have demonstrated that chemotactic characteristics of defensins on various immune cells: HNP1–3 and HBD3-4 for monocytes/macrophages (Territo *et al.*, 1989; Verbanac *et al.*, 1993; Yang *et al.*, 2002), HBD2 for neutrophils activated by tumor necrosis factor (TNF)-alpha (Niyonsaba *et al.*, 2004), HNP1, HBD1–3 for immature DCs (Yang *et al.*, 2000; Conejo-Garcia *et al.*, 2004), HNP1-2 for peripheral blood T cells

(Chertov et al., 1996; Yang et al., 2000) and HBD1 and HBD2 for memory T cells expressing CCR6 (Yang et al., 1999). The chemoattractive effect of defensins on T cells suggests that they can help the recruitment of $CD4^+$ and $CD8^+$ effector T cells to the site of microbial infection (Yang et al., 2000).

Direct regulation of defensins expression

The production of defensins is induced by stimuli sensed by receptors called pattern recognition receptors (PRRs) on the surface of defensin-expressing cells. The stimuli include various microbial products, such as proteins, lipids, lipoproteins and nucleic acids (Tang et al., 2012). There are four types of PRR: the toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, retinoic acid-inducible gene-I-like receptors and C-type lectin receptors (Takeuchi and Akira, 2010). Corresponding ligand binding of PRRs triggers the subsequent signaling cascade, resulting in the activation of transcription factors that target the gene transcription of defensins or other antimicrobial peptides in response to microbial stimuli or other physical, physiological or oxidative stressors (Oeckinghaus et al., 2011). The release of HNP1–3 by neutrophils can be triggered in response to chemokines, FCγ receptor cross-linking, phorbol myristate acetate and bacterial products binding TLRs (Fruitwala et al., 2019). NOD2 and Wnt signaling transcription factor TCF-4 (T cell factor 4) proteins regulate the expression of HD5 in the small intestine (Wehkamp et al., 2004, 2007). HD6 gene expression is influenced by the TCF-4 binding site and the E-box site in the proximal promoter region (Hayashi et al., 2016). The expression of HBD1–3 is mainly induced by TLRs (Fruitwala et al., 2019).

Defensins expression and hormonal regulation in the human reproductive tracts

Defensins expression and hormonal regulation in the female reproductive tract

The FRT is constantly challenged by exogenous pathogens; however, during pregnancy, the FRT immune response must distinguish between the developing fetus and evading pathogens to protect the former and eliminate the latter. In accordance with these double roles, the FRT is divided into the upper FRT (including the endocervix, uterus and fallopian tubes) and the lower FRT (including the vagina and ectocervix) (Wira et al., 2015). The upper FRT is lined by simple columnar epithelium, and the main immune cell population in the lamina propria is composed of T cells, macrophages and innate lymphoid cells (ILCs, including natural killer cells, ILC1, ILC2, ILC3 and lymphoid tissue-inducer (LTi) cells) (Vivier et al., 2018). In contrast, the lower FRT is lined by non-keratinized stratified squamous epithelium, and the immune cell population in the lamina propria is more diverse, including T cells, DCs, granulocytes, macrophages, ILCs and B cells (Iwasaki, 2010; Monin et al., 2020). While the composition of immune cells changes under the regulation of hormones during the menstrual cycle in the upper FRT, it remains relatively stable in the lower FRT. However, their function may differ during the menstrual cycle (Wira et al., 2015). On the inner surface of the FRT epithelium, a layer of mucus made up of mucins and containing many antimicrobial factors, like complement components and antimicrobial peptides, forms a chemical barrier for the FRT (Iwasaki, 2010). As important members of the antimicrobial peptides, α and β-defensins are produced by leucocytes or epithelial cells distributed along the FRT, with different preferences.

α-defensins

Transcriptome data (GEO accession No.: GSE7307) show that the levels of mRNA (*DEFA1–3*) encoding HNP1–3 in the lower FRT (vagina) are higher than in the other parts of the FRT (Fig. 2A, Table I). This is due to the ability of most of the immune cells in the vaginal lamina propria to produce and secrete HNP1–3, including neutrophils, eosinophils, natural killer cells, monocytes, γδT lymphocytes, B lymphocytes and immature DCs (Agerberth et al., 2000; Nathan, 2006; Rodríguez-García et al., 2007; Driss et al., 2009). HNP1–3 have also been tested via ELISA in cervicovaginal secretions and are regarded as an indicator of FRT viral and bacterial infection (Shust et al., 2010; Levinson et al., 2012; Guthrie et al., 2015). Moreover, HNP1–3 are expressed in the endometrium and play a role in maternal antibacterial process (as discussed below) (Das et al., 2007; King et al., 2007a,b). Although the mRNA encoding HNP4 was detected at relatively high levels in the vulva, vagina, cervix and uterus, and at low levels in the endometrium, myometrium, and ovary via the GeneChip array, the protein has not been tested in any research so far. Both the mRNA and protein for HD5 have been verified in FRT. It is constantly expressed in the upper half of the stratified squamous epithelium of the vagina and ectocervix with an increased amount toward the lumen and is also present in the endocervix, endometrium and fallopian tube with an individual differences (Quayle et al., 1998). The transcriptome data of endometrium and myometrium and *in vitro* studies using immortalized epithelial cells from normal human vagina, ectocervix and endocervix have reported the presence of mRNA encoding HD6 (Fichorova et al., 1997; Klotman et al., 2008). However, the HD6 protein has not been verified in the FRT so far. Also, in contrast with the transcriptome data where HNP4, HD5 and HD6 had relatively high mRNA levels in the vulva, Erhart et al. (2011) demonstrated scarcely detectable HNP1–3, HD5 and HD6 in normal or infected vulval tissue using QPCR (Quantitative real-time polymerase chain reaction) and immunohistochemical analysis.

β-defensins

Among the 11 β-defensins studied at the protein level (see Table I), only HBD1–4 have been exclusively studied in the FRT (Fig. 2B). All four proteins are mainly expressed in the corresponding epithelial cells. HBD1 and HBD2 are expressed along the upper and lower FRT and in the cervicovaginal secretions, and the expression can be upregulated upon infection status (Valore et al., 1998; King et al., 2003a; Ghosh et al., 2008; Meng et al., 2013). HBD3 mRNA or protein has been reported in the vagina, cervix and endometrium in large amounts; however, they are not present in the fallopian tube (King et al., 2003a; Meng et al., 2013; Mitchell et al., 2013). In contrast, HBD4 is only expressed in the endometrium (King et al., 2003b).

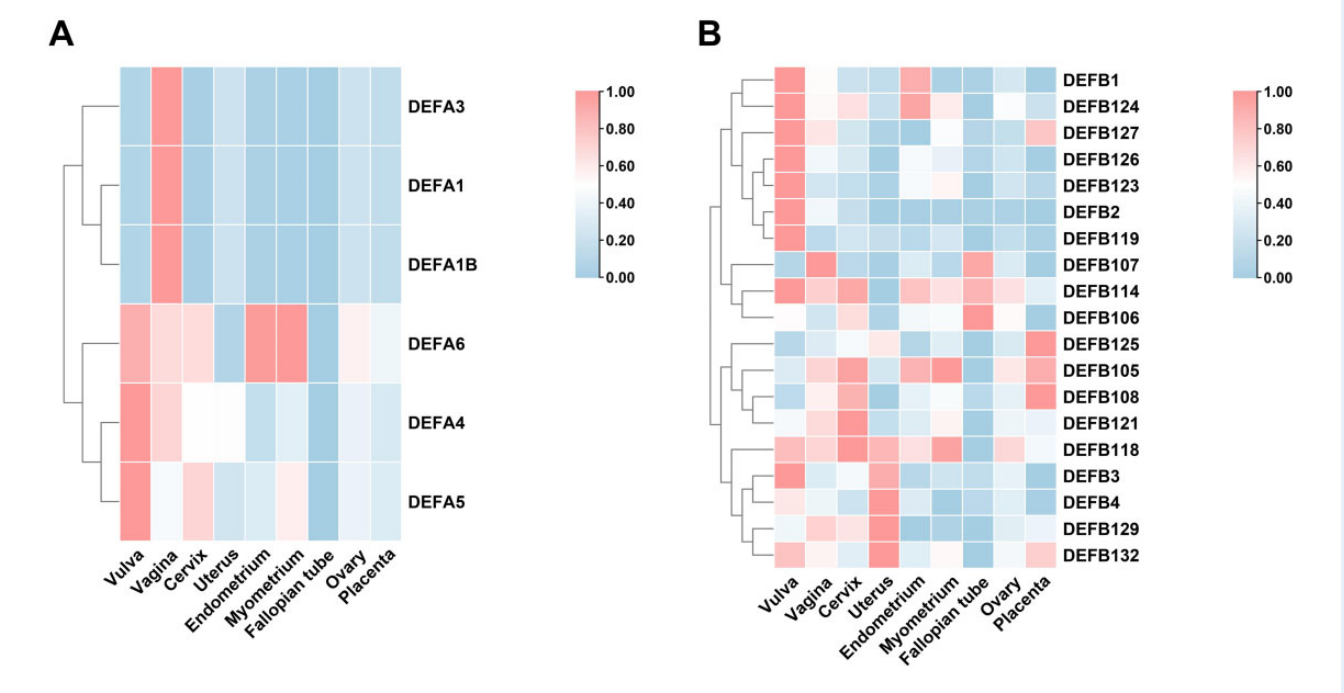


Figure 2. Transcriptome data of defensins in the female reproductive tract. (**A**) α-defensins. (**B**) β-defensins. The mean mRNA levels of 6 α-defensins and 18 β-defensins in the MRT (including vulva, vagina, cervix, uterus, endometrium, myometrium, fallopian tube, ovary and placenta) were summarized from transcriptome data GSE7307 in the GEO database. Number of samples: 4 vulvae, 4 vaginae, 4 cervices, 1 uterus, 18 endometria, 19 myometria, 1 fallopian tube, 5 ovaries and 1 placenta.

Hormonal regulation of defensins expression during the menstrual cycle

The innate and adaptive immune responses in the FRT are greatly affected by the hypothalamic–pituitary–ovarian axis. The immune responsive protection in the FRT is downregulated during the secretory phase, which is also called a 'window of vulnerability', to allow the passage of 'non-self' sperm for fertilization, implantation and pregnancy (Yarbrough *et al.*, 2015). Hence, apart from the sectional distribution of defensins in the FRT, the levels of defensins vary during the menstrual cycle. In the cervicovaginal secretions, the protein levels of secretory HNP1–3 and HBD3 are maximal in the secretory phase (Shust *et al.*, 2010). In the endometrium, maximal mRNAs encoding different defensins have been monitored (Fig. 3): HBD3 in the secretory phase (King *et al.*, 2003b), HD5 in the early secretory phase (Quayle *et al.*, 1998) and HBD1 in the late secretory and menstrual phase (Fleming *et al.*, 2003). The secretory HBD4 was maximal in the proliferative phase (King *et al.*, 2003b) and HBD2 was maximal in the menstrual phase (Fleming *et al.*, 2003). Moreover, human immunodeficiency virus (HIV)-negative postmenopausal women had significantly lower levels of HBD2 compared with HIV-negative premenopausal women (Ghosh *et al.*, 2019). Hormonal contraception can suppress natural antimicrobial gene transcription in the human endometrium (Fleming *et al.*, 2003). The levels of defensins were decreased in the cervicovaginal lavage of women on hormonal contraception (Shust *et al.*, 2010). With progesterone treatment to prevent preterm birth, the vaginal progesterone treatment can increase the expression of HBD1 (Nold *et al.*, 2013). Thus, both endogenous and exogenous hormones affect the expression of defensins. The underlying mechanism of hormonal regulation of FRT defensins is mainly related to the varied expression of PRRs, like TLRs, during the menstrual cycle (Wira *et al.*, 2015).

Defensins expression and hormonal regulation in the male reproductive tract

The male reproductive system includes the testes, excretory genital ducts (epididymis, ductus deferens and ejaculatory duct), accessory glands (seminal vesicles, prostate glands and bulbourethral glands) and penis (urethra within). Seminiferous tubules in the testes produce spermatozoa which subsequently mature in the epididymis to acquire motility and capacitation for the later fertilization process. The genital ducts and accessory glands produce a mixture of secretions to form the seminal plasma. Upon ejaculation, spermatozoa are propelled through the vas deferens into the urethra in conjunction with seminal plasma to form the semen (a suspension of spermatozoa) for ejaculation through the penile urethra. Hence, the major function of the male reproductive system is to produce viable male germ cells to deposit in the FRT and finally complete fertilization (Mawhinney and Mariotti, 2013). The MRT is also constantly facing the challenge of external insults. However, due to the distinct mucosal environment, the distribution of defensins along the MRT is quite different from the situation in the FRT. Androgen receptor (AR) binding sites have been found in β-defensin promoters or intronic regions in mouse epididymis, indicating a direct hormonal regulation of the protein expression (Hu *et al.*, 2014). Various β-defensins have been found to be preferentially

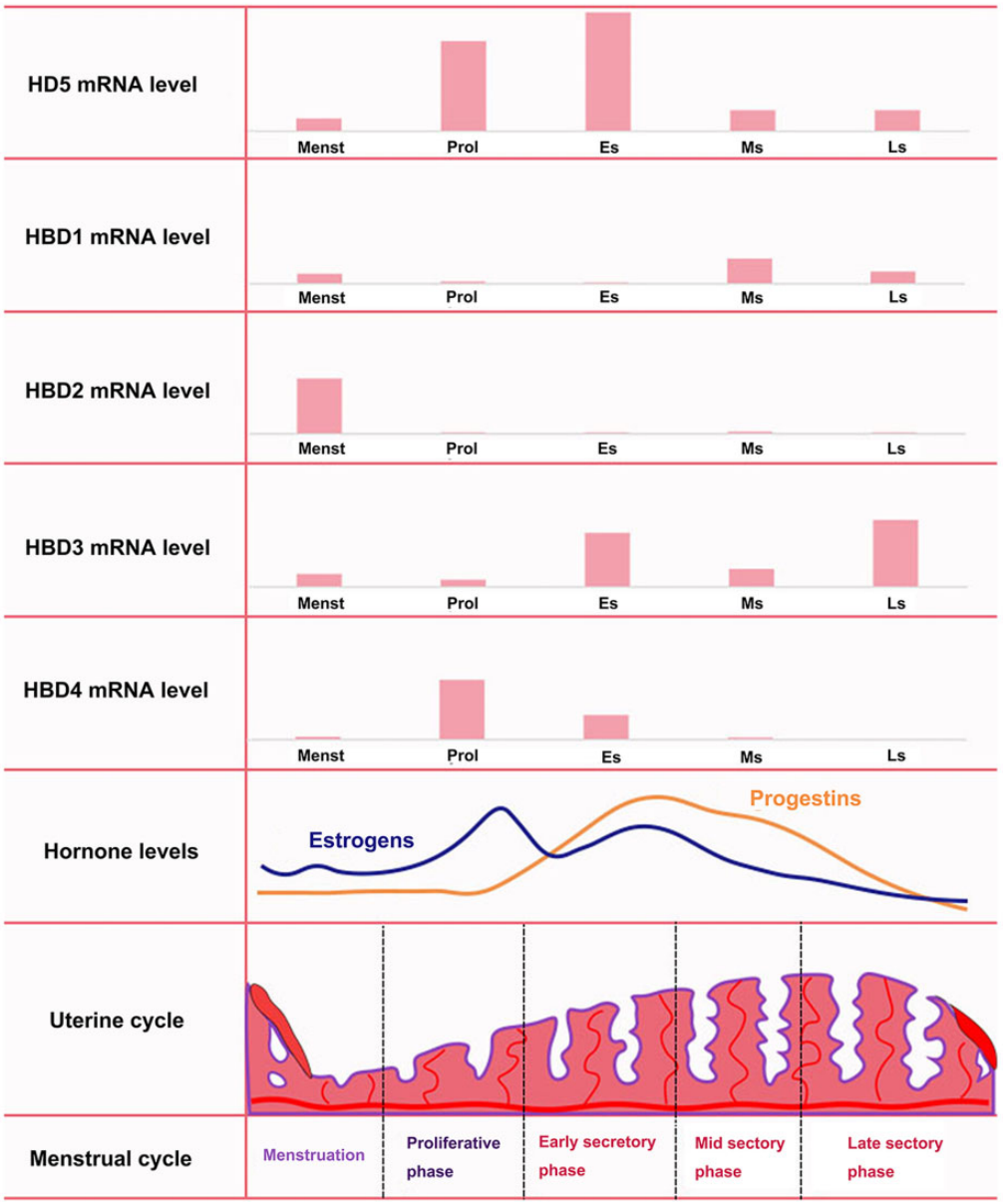


Figure 3. mRNA expression of defensins in the endometrium changes during the menstrual cycle. Menst, menstrual phase; Prol, proliferative phase; Es, early secretory phase; Ms, mid secretory phase; Ls, late secretory phase.

expressed in the male reproductive system, especially in the testis and epididymis (Yamaguchi *et al.*, 2002; Zaballos *et al.*, 2004; Patil *et al.*, 2005). In addition, many β-defensins in epididymis and testis are developmentally regulated, with elevated expression during puberty and the sexual maturation period (Sangeeta and Yenugu, 2019).

α-defensins

Published experimental data on the distribution and protein levels of α-defensins in the MRT are limited. The transcriptome data in the GEO database (GEO accession No.: GSE150852) (Robertson *et al.*, 2020) showed very low levels of mRNAs encoding HNP1–3 in the testis and a relatively high level of mRNA encoding HNP3 in the epididymal caput and cauda (Fig. 4A). Com *et al.* (2003) reported low levels of mRNAs encoding HNP1–3, HD5 and HD6 in human testis and high levels of mRNAs encoding HNP1–3 mRNA but not HD5 and HD6 in normal spermatozoa isolated from semen (Table I). The presence of HNP1–3 in testis tissue may come from the large numbers of macrophages and lymphocytes in the interstitial tissue of the testis. Moreover, the leucocytes may contribute to the high level of HNP1–3 in semen isolated spermatozoa due to 'round cell'

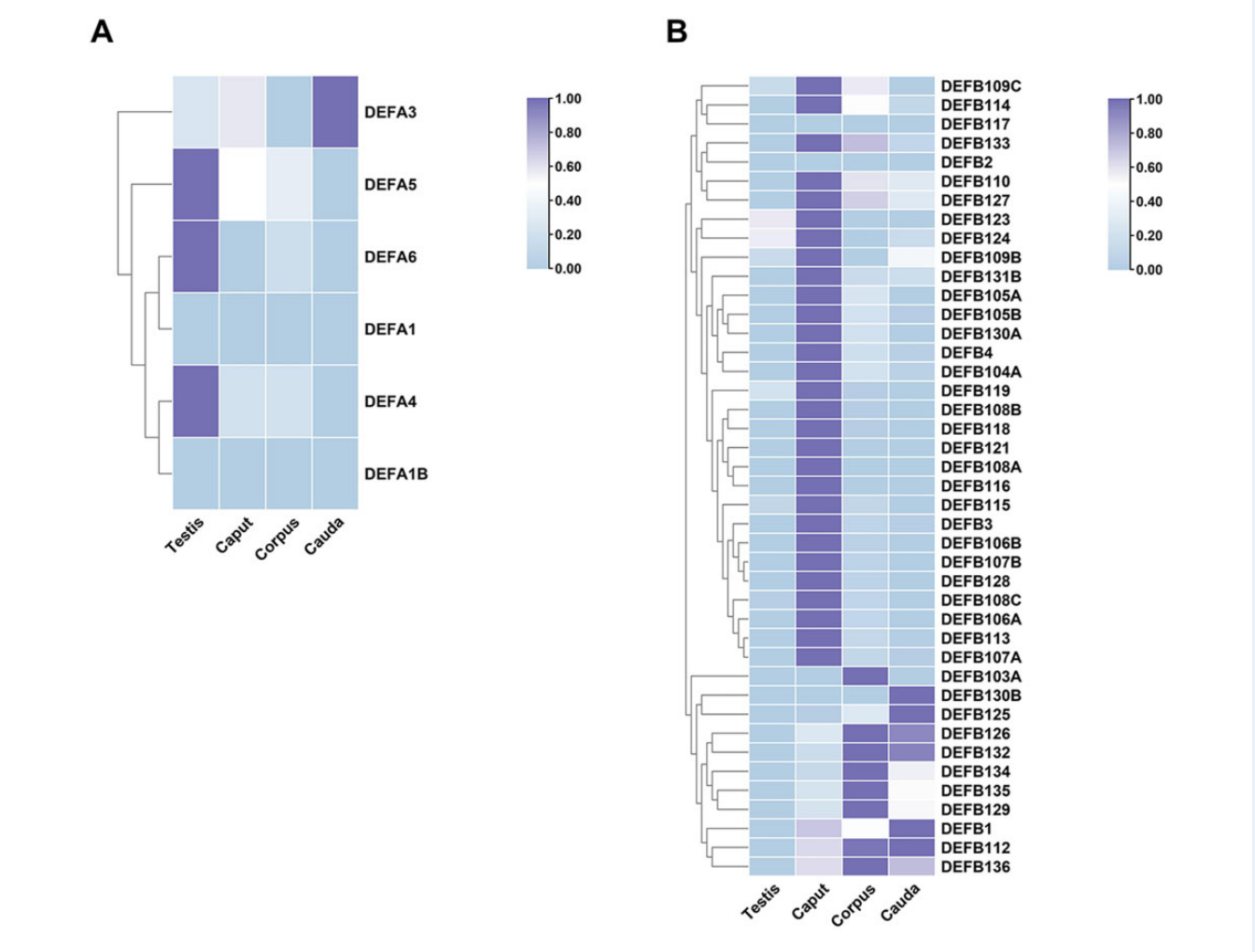


Figure 4. Transcriptome data of defensins in the male reproductive tract. (**A**) α-defensins. (**B**) β-defensins. The mean mRNA levels of 6 α-defensins and 41 β-defensins in the testis and the caput, corpus and cauda of the epididymis were summarized from transcriptome data GSE150852 in the GEO database. Number of samples: 3 testes, 3 capita, 3 corpora and 3 caudae.

contamination (Barratt *et al.*, 1990). However, we did not find published data on HNP1–3, HD5 and HD6 in the epididymis, ductus deferens, ejaculatory duct and urethra. Several studies have reported the acute inflammatory role and inhibition effect of neutrophil-origin α-defensins on prostate cancer (Sfanos *et al.*, 2009; Kohli *et al.*, 2015; Sasani *et al.*, 2021).

β-defensins

Unlike the well-studied situation in the FRT, the research data on HBD1–4 in the MRT are very limited. HBD1 mRNA and protein were detected in human testis tissue, spermatozoa, semen and prostate gland (Table I) (Zhao *et al.*, 1996; Com *et al.*, 2003; Hong *et al.*, 2017; Zupin *et al.*, 2019a). The expression of HBD2 was not found in the transcriptome data or published research. Although mRNA encoding HBD3 was high in the transcriptome data, we did not find published results consistent with this either. The presence of HBD4 was confirmed at the mRNA level in human epididymis (Yamaguchi *et al.*, 2002). Notably, the transcriptome data showed high mRNA levels for 39 out of 42 β-defensins listed in the data source (Fig. 4B) in the epididymal caput, corpus and cauda but not in the testis. Over the years, many novel β-defensins have been identified in the male reproductive system in different mammal species (reviewed in Hall *et al.*, 2007; Tollner *et al.*, 2012; Dorin and Barratt, 2014). In humans, *DEFB129* mRNA was reported in the testis (Miyai *et al.*, 2021); the proteins DEFB106A, DEFB114 and DEFB129 were detected in the epididymis (Dubé *et al.*, 2008; Yu *et al.*, 2013; Xin *et al.*, 2014); the proteins DEFB118 and DEFB126 were found on sperm (Yenugu *et al.*, 2004; Aram *et al.*, 2020); DEFB132 (also called HEL-75) protein was detected in both epididymis and sperm (Lin *et al.*, 2008); and DEFB124 protein was found in the prostate epithelia (Kim *et al.*, 2014).

Hormonal regulation of defensins expression in MRT

The expression of defensins in the MRT varies with androgen levels and ages. Since the androgen/AR signaling pathway plays an important

regulatory role in the structure and function of the epididymis, many epididymal secretory proteins involved in sperm maturation have been identified as androgen regulatory proteins, including some β-defensins (Patrão *et al.*, 2009). For example, the expression of human DFEB118 is regulated by androgen level (Yenugu *et al.*, 2004). Due to limited access to human epididymal samples or effective *in vitro* human epididymal cell models, we did not find published results on the androgen regulation mechanism on human defensins. Most mechanistic research has been done on model animals such as mice and rats. Rat epididymis-specific β-defensin 15 (Defb15) exhibits an androgen-dependent expression pattern (Zhao *et al.*, 2011). In mice, some β-defensins, including Spag11a (Bin1b), Defb20, 22, 41 and 42, are regulated by androgens (Jalkanen *et al.*, 2005; Oh *et al.*, 2006; Hu *et al.*, 2010; Pujianto *et al.*, 2013). The androgen/AR differentially regulates β-defensins in the epididymal caput of mice (Hu *et al.*, 2014). In addition to androgens and testicular factors can regulate some β-defensins (Hu *et al.*, 2014; Pujianto *et al.*, 2020). The destruction of the hypothalamic–pituitary testis axis changed defensin expression in the boar testis and epididymis (Weber *et al.*, 2018). In addition, there are differences in the expression profiles of defensin proteins in the epididymis and testis of extreme age rats (Sangeeta and Yenugu, 2019), which may be due to the different physiological conditions of rats and the difference in serum testosterone content.

Host defence by defensins in the reproductive system

Microbial-induced reproductive tract diseases, including sexually transmitted infections (STIs), bring huge burdens to human reproductive health by escaping the protection conferred by the mucosal immune system. Defensins play important roles in protecting the reproductive tracts from microbial invasion (Table II).

Bacteria

Gardnerella vaginalis

Gardnerella vaginalis infection can cause bacterial vaginosis (BV), leading to an imbalanced vaginal microbiota (Mendling, 2016). The loss of normal immunostimulatory flora in BV is associated with a local deficiency of multiple innate immune factors that may predispose individuals to STIs (Valore *et al.*, 2006). Clinical studies have found defensin deficiency in vaginal lavage fluids of women with BV. Effective treatment normalizes defensin levels in BV, suggesting that abnormal defensin levels are a consequence of *G. vaginalis* infection (Balu *et al.*, 2002; Valore *et al.*, 2006; Fan *et al.*, 2008; Libby *et al.*, 2008; Xu *et al.*, 2008; Mitchell *et al.*, 2013; Noda-Nicolau *et al.*, 2021). However, *in vitro* experiments showed that *G. vaginalis* infection could induce HBD1–3 secretion in human chorioamnion (Zaga-Clavellina *et al.*, 2012a).

Inconsistent changes in defensin levels were found in lavage fluids from patients with *G. vaginalis* infection, for instance, decreased levels of HBD1–3 in the cervix of childbearing age women (Noda-Nicolau *et al.*, 2021), increased HNP1–3 concentrations in vaginal fluid of pregnant women (Balu *et al.*, 2002; Xu *et al.*, 2008) and low HBD3 concentrations in vaginal fluid of African American pregnant women (Mitchell *et al.*, 2013). In postmenopausal women, HBD1 levels were significantly decreased, and HBD2 and HBD3 levels were significantly increased (Brunner *et al.*, 2021). The inconsistent changes in defensins may be due to the inability of the studies described above to standardize the collection of vaginal effusions, the differences in the physiologic status of the study subjects, and the differences in the immune defence systems between non-pregnant and pregnant women, as well as the effect of ethnic differences.

Escherichia coli, Chlamydia trachomatis and *Neisseria gonorrhoeae*

The Gram-negative bacteria, *Escherichia coli*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, are the most common causes of urogenital infections (Mcconaghy and Panchal, 2016). When sexually transmitted, these bacteria can cause genitourinary tract infections, such as urethritis or cervicitis, in women (whereas salpingitis is a more serious complication) and urethritis combined with epididymitis or proctitis, in men. The pathogenicity of Gram-negative bacteria is usually related to a major component of their cell walls, specifically a lipopolysaccharide (also known as LPS or endotoxin) layer. In humans, LPS can stimulate an innate immune response (Silva *et al.*, 2018), including inducing the expression of defensins in the reproductive tract. Defensins regulate the immune response of macrophages against Gram-negative bacterial infection by neutralizing LPS (Wilson *et al.*, 2013).

Infections with *C. trachomatis* and *N. gonorrhoeae* cause abnormal expression of defensins in the female reproductive system. Noda-Nicolau *et al.* (2017) found that the levels of HBD1, HBD2 and HBD3 in the cervicovaginal fluid of *C. trachomatis*-positive women were significantly lower than those of negative women. Wiechuła *et al.* (2010) found that *C. trachomatis* infection activates HBD2 production but causes variable increases in HBD1 concentrations. Selective stimulation of the placental outer membrane by *E. coli* results in tissue-specific secretion of HBD1, HBD2 and HBD3 primarily in the choriodecidual, the first area of infection during ascending infection. Among the three defensins, HBD3 was a potential candidate for enhancing cervical innate immunity to prevent ascending infection-related preterm birth and its associated neonatal consequences (Garcia-Lopez *et al.*, 2010). HD5 and HNP1–3 were significantly elevated in male urethral and female vaginal secretions with *C. trachomatis* and *N. gonorrhoeae*. The HD5-mediated antibacterial activity against *C. trachomatis* depends on its N-terminal processing modification (Porter *et al.*, 2005). *In vitro* studies involving the treatment of cells with the pro-inflammatory factor LPS can upregulate the expression and production of HBD2 in primary endometrial epithelial cells (Wilson *et al.*, 2013), vaginal epithelial cell lines (Pivarcsi *et al.*, 2005) and human amniotic epithelial cells (Flores-Espinosa *et al.*, 2014).

There are many studies related to LPS and male reproduction. LPS induces epididymitis and affects sperm motility. Experiments show that LPS can upregulate tissue concentrations of inflammatory mediators and cytokines/chemokines, thereby activating acute inflammation and reducing epididymal sperm count and transit time (Silva *et al.*, 2018). LPS infection also affects the expression of epididymal defensin. In LPS-induced rat epididymitis, the gene expression of Defb2, Defb21 and Defb27 was decreased in the epididymal head (Biswas and Yenugu, 2011). Sperm-associated antigen 11e (Spag11e), an epididymal-specific defensin, is downregulated and simultaneously reduces sperm function parameters (Cao *et al.*, 2010). Conversely, some studies found that LPS induced the expression of defensins, Spag11e and defensin-like Spag11 genes in male rat reproductive tissues (Biswas and Yenugu, 2011, 2013, 2014). The DEFB126 core

Table II Defensins against reproductive tract infection.

Microorganism[1]	Female reproduction[2]		Male reproduction[3]	
	Defensin[4]	Reference	Defensin[4]	Reference
HIV (Human Immunodeficiency Virus)	HNP1, HNP2, HNP3, HD5 and HD6	(Klotman *et al.*, 2008; Levinson *et al.*, 2009; Pellett Madan *et al.*, 2015)	α-defensin	(Hirbod *et al.*, 2014; Prodger *et al.*, 2014)
	HBD1, HBD2 and HBD3	(Ricci *et al.*, 2009; Aguilar-Jiménez *et al.*, 2011; Jiang *et al.*, 2012; Hughes *et al.*, 2016; Jais *et al.*, 2016; Zupin *et al.*, 2019b)	HBD2 and HBD3	(Kreuter *et al.*, 2009)
	θ-defensin (DEFT)	(Yang *et al.*, 2005)	θ-defensin	Not reported
HPV (Human Papilloma Virus)	HNP1 and HD5	(Hubert *et al.*, 2014; Zhao *et al.*, 2015)	α-defensin	Not reported
	HBD1, HBD2, HBD3 and HBD4	(Erhart *et al.*, 2011; Abe *et al.*, 2013; Segat *et al.*, 2014; Buckley *et al.*, 2016; Szukiewicz *et al.*, 2016)	HBD2 and HBD3	(Kreuter *et al.*, 2009)
	θ-defensin	Not reported	θ-defensin	Not reported
HSV (Herpes Simplex Virus)	HNP1, HNP2 and HNP3	(John *et al.*, 2005)	α-defensin	Not reported
	HBD1 and HBD2	(Keller *et al.*, 2019; Fichorova *et al.*, 2020)	β-defensin	Not reported
	θ-defensin	Not reported	θ-defensin	Not reported
Neisseria gonorrhoeae	HNP1, HNP2, HNP3, HD5 and HD6	(Wiesenfeld *et al.*, 2002; Klotman *et al.*, 2008)	HNP1, HNP2, HNP3, and HD5	(Porter *et al.*, 2005)
	β-defensin	Not reported	β-defensin	Not reported
	θ-defensin	Not reported	θ-defensin	Not reported
Chlamydia trachomatis	HNP1, HNP2 and HNP3	(Wiesenfeld *et al.*, 2002; Wiechuła *et al.*, 2007)	HNP1, HNP2, HNP3, and HD5	(Porter *et al.*, 2005)
	HBD1, HBD2, HBD3 and HBD4	(Wiechuła *et al.*, 2010; Mukura *et al.*, 2017; Noda-Nicolau *et al.*, 2017; Fichorova *et al.*, 2020)	β-defensin	Not reported
	θ-defensin	Not reported	θ-defensin	Not reported
Gardnerella vaginalis	HNP1, HNP2, HNP3 and HD5	(Sawada *et al.*, 2006; Fan *et al.*, 2008; Xu *et al.*, 2008)	α-defensin	Not reported
	HBD1, HBD2 and HBD3	(Balu *et al.*, 2002; Valore *et al.*, 2006; Fan *et al.*, 2008; Eade *et al.*, 2012; Jiang *et al.*, 2012; Zaga-Clavellina *et al.*, 2012a; Mitchell *et al.*, 2013; Castro *et al.*, 2018; Fichorova *et al.*, 2020; Noda-Nicolau *et al.*, 2021)	β-defensin	Not reported
	θ-defensin	Not reported	θ-defensin	Not reported
Escherichia coli	α-defensin	Not reported	Recombinant defensin-21	(Biswas *et al.*, 2015)
	HBD1, HBD2 and HBD3	(Garcia-Lopez *et al.*, 2010; Ghartey *et al.*, 2012; Suff *et al.*, 2020)	DEFB118 and DEFB114	(Yenugu *et al.*, 2004; Hou *et al.*, 2021) (Yu *et al.*, 2013)
			cBD-1, cBD-2, and cBD-3	(Sang *et al.*, 2005)
			rBD-1, rBD-2, rBD-22, and rBD-42	(Palladino *et al.*, 2003; Diao *et al.*, 2011; Xin *et al.*, 2015)
			mBin1b	(Li *et al.*, 2001; Fei *et al.*, 2012)

(continued)

Table II Continued

Microorganism[1]	Female reproduction[2]		Male reproduction[3]	
	Defensin[4]	Reference	Defensin[4]	Reference
Candida	α-defensin	Not reported	α-defensin	Not reported
	HBD1, HBD2 and HBD3	(Pivarcsi *et al.*, 2005; Wiechuła *et al.*, 2010; Zaga-Clavellina *et al.*, 2012b; Fichorova *et al.*, 2020; Kotani *et al.*, 2020; Miró *et al.*, 2021)	DEFB114	(Yu *et al.*, 2013)
	mBD-1	(Miró *et al.*, 2017)		
	θ-defensin	Not reported	θ-defensin	Not reported
Ureaplasma	α-defensin	Not reported	α-defensin	Not reported
	HBD3	(Tantengco *et al.*, 2021)	β-defensin	Not reported
	θ-defensin	Not reported	θ-defensin	Not reported
Trichomonas vaginalis	HNP1, HNP2 and HNP3	(Wiesenfeld *et al.*, 2002)	α-defensin	Not reported
	HBD2	(Fichorova *et al.*, 2015)	β-defensin	Not reported
	θ-defensin	Not reported	θ-defensin	Not reported

The different colors summarize different defensins. The yellow is for α-defensins, the blue is for β-defensins and the green is for θ-defensin.
[1]Microorganism associated with male and female reproductive tract infections.
[2]Research on defensins related to female reproduction, germ-related cell lines, germ-related tissues, germ-related organs and germ-related secretions were included.
[3]Research on defensins-related male reproduction, germ-related cell lines, germ-related tissues, germ-related organs and germ-related secretions were included.
[4]Human neutrophil defensins (HNP), human α-defensin 5 (HD5), human α-defensin 6 (HD6), human β-defensin (HBD), canine β-defensin (cBD), rat β-defensin (rBD), mouse β-defensin (mBD), mBin1b: a β-defensin gene identified in the mouse epididymis.

peptide binds to LPS to exert anti-inflammatory and intracellular regulatory functions (Liu *et al.*, 2013). Truncated α-defensin analogues have inhibitory and killing activities against multidrug-resistant *E. coli* (UPEC) biofilmsin an *in vitro* study (Moazzezy *et al.*, 2020). The conserved cysteine and arginine residues of HD5 are indispensable for its antibacterial and LPS neutralizing effects (Chen *et al.*, 2019). In addition, the core peptide of a novel antimicrobial peptide DEFB118 significantly inhibited the mRNA levels of LPS-induced inflammatory cytokines, including IL-α, IL-1β, IL-6 and TNF-α, in RAW264.7 cells, correspondingly decreasing the secretion of IL-6 and TNF-α (Lin *et al.*, 2020; Hou *et al.*, 2021). Defensins can inhibit LPS-induced inflammation and promote sperm motility. When male mice were infected by uropathogenic UPEC, both mBD-3 and mBD-14 exhibited dose-dependent bactericidal activity against UPEC (Becknell *et al.*, 2013). Recombinant defensin 21 reduces the bacterial load in the rat epididymis and testis more effectively than gentamicin (Biswas *et al.*, 2015). Recombinant human DEFB114 rescues human sperm motility through its LPS-neutralizing activity (Yu *et al.*, 2013).

Virus

Human immunodeficiency virus

The main routes of HIV transmission are sexual transmission, blood transmission and mother-to-child transmission (Pope and Haase, 2003; Page-Shafer *et al.*, 2006). HIV specifically invades and destroys helper T lymphocytes and, at the same time, damages a variety of immune cells in the human body. The invasion is eventually complicated by various serious opportunistic infections and malignant tumors, which are called acquired immunodeficiency syndrome (Appay and Sauce, 2008). Defensins influence HIV transmission, replication and disease progression through their lectin properties, immune regulation, genetic polymorphisms and copy number variation (CNV), etc.

The α-defensins fight HIV infection mainly through lectin properties, fusion inhibition and viral replication. The anti-HIV activity of HNP1–3 depends on its lectin properties. $CD4^+$ T cells can be protected from HIV-1 infection by these α-defensins binding to the sugar moiety on CD4 and the viral envelope glycoprotein gp120 inhibiting viral entry (Wang *et al.*, 2004; Furci *et al.*, 2007; Demirkhanyan *et al.*, 2012). Therefore, the antiviral activity of HNP1–3 may be disrupted by glycosylated serum proteins. However, in the presence of serum, HNP1 can act on cells to interfere with the PKC signaling in primary $CD4^+$ T cells, thereby inhibiting HIV-1 replication (Chang *et al.*, 2005). HNP4 is more potent than HNP1–3 in protecting human peripheral blood mononuclear cells from X4 and R5 HIV-1 strains, but its anti-HIV activity is independent of lectin properties (Wang *et al.*, 2004). *In vitro* studies showed that HD5 inhibited HIV-1 entry into target cells at physiological concentrations in a serum-free environment by interfering with the interaction between gp120 and CD4, which is similar to the mechanism of the HNPs, probably due to a high degree of structural homology (Furci *et al.*, 2012). Furthermore, HD5 was significantly more effective than HNP1 in downregulating the CXC chemokine receptor 4 (CXCR4) in activated primary T cells, which is the primary target of HIV-1 infection. Reduction of cell surface CXCR4 has been reported to significantly inhibit X4 HIV-1-mediated fusion efficiency and viral replication (Zhou *et al.*, 2004b; Anderson and Akkina, 2005);

thus, CXCR4 regulation represents another possible mechanism for HD5-mediated HIV-1 inhibition at virus entry level.

However, contradictory pieces of evidence also exist. HD5 and HD6 were reported to enhance HIV-1 infectivity by promoting virus attachment (Ellegård *et al.*, 2011; Ding *et al.*, 2013). HD5 and HD6 are elevated in the genital mucosa of individuals with STIs. And HD5 has been shown to contribute to the STI-mediated increase in HIV infectivity *in vitro* (Klotman *et al.*, 2008; Valere *et al.*, 2015). This may be related to the elevated concentrations of HD5 and HD6 upon STIs infection. It is possible that at high concentrations, HD5 and HD6 form cationic aggregates that enhance HIV-1 infectivity by neutralizing membrane charges or promoting viral aggregation (Rapista *et al.*, 2011). Furthermore, the enhancement of HIV by HD5 and HD6 is influenced by peptide structure, as loss of intramolecular cysteine linkages is associated with loss of HIV enhancement (Klotman *et al.*, 2008). The mutation of residues involved in the hydrophobicity and self-association of defensins significantly deprives the ability of HD5 and HD6 to promote HIV attachment and infection (Valere *et al.*, 2017).

Polymorphisms and CNV in β-defensin genes are associated with susceptibility to HIV infection and disease progression. Three single-nucleotide polymorphisms in the 5′ untranslated region of the *DEFB1* gene, namely -52(G/A), -44(C/G) and -20(G/A), can be used as markers of HIV-1 infection risk and vary by population (Milanese *et al.*, 2006; Baroncelli *et al.*, 2008; Segat *et al.*, 2009; Zupin *et al.*, 2019b). In Brazilian HIV-positive children, the median copy number of *DEFB104* was lower than HIV-exposed, uninfected children and healthy controls, suggesting that *DEFB104* CNV may have been involved in protection from vertical transmission of HIV (Milanese *et al.*, 2009). A North American, predominantly white, cohort observed that higher *DEFB4/103A* CNV was associated with slower progression to acquired immune deficiency syndrome (AIDS) (Mehlotra *et al.*, 2012). The β-defensin gene copy number results for 1002 Ethiopian and Tanzanian patients show that higher β-defensin CNV was associated with increased HIV load before highly active antiretroviral therapy (HAART) ($P = 0.005$) and poor immune reconstitution after HAART initiation ($P = 0.003$) (Hardwick *et al.*, 2012).

β-defensins inhibit HIV infection through immunomodulatory effects. HBD3 blocks HIV infection through direct interaction with virions and by acting as an antagonist of CXCR4 on T cells. HBD3 competes with the natural ligand of CXCR4, stromal-derived factor 1 (SDF-1), for cell binding and blocks CXCR4 activation (Feng *et al.*, 2006). The ability of HBD3 to inhibit the SDF-1α-CXCR4 interaction is related to the presence of HBD3 cysteine residues, the specific surface distribution of cationic residues, and the electrostatic properties and availability of both HBD3 termini (Feng *et al.*, 2013). Additionally, *in vitro*, in primary human monocyte-derived macrophages, HBD2 and HBD3 inhibit HIV replication in a dose-dependent manner (Bharucha *et al.*, 2021). HBD3 also induces chemokines in monocytes and macrophages, allowing cells to reach sites of inflammation (Petrov *et al.*, 2013). HBD2 suppresses HIV at an early post-entry stage via G-protein coupled receptor-mediated signaling (Bharucha *et al.*, 2021) and selectively protects primary $CCR6^{+}CD4^{+}$T cells infected with HIV-1 (Lafferty *et al.*, 2017).

The synthetic human-derived θ-defensin polypeptides similar to rhesus theta-defensins (RTDs), 'retrocyclin', were identified as a potential novel small molecule capable of inhibiting HIV-1 entry and fusion (Münk *et al.*, 2003; Penberthy *et al.*, 2011). θ-defensins have lectin properties as well as immunomodulatory effects. Retrocyclin has a high affinity for the HIV-1 glycoproteins gp120 and gp41 and the host cell glycoprotein CD4; therefore, it can inhibit the fusion of virus and target T cells (Münk *et al.*, 2003; Wang *et al.*, 2003). RTD-1 can also downregulate CXCR4 and inhibit HIV replication (Seidel *et al.*, 2010). In addition, retrocyclins can block infection by inhibiting HIV-1 fusion with host cells. After HIV-1 gp120 binds to its receptor and co-receptor, the outer domain of gp41 transmembrane protein undergoes a significant conformational change to form a membrane-penetrating six-helix bundle required for host cell membrane fusion. Retrocyclins can prevent HIV-1 fusion with host cells by binding to gp41 and blocking the six-helix bundle formation (Cole *et al.*, 2006; Gallo *et al.*, 2006).

θ-defensins are currently considered the greatest clinical potential as biocides of HIV fusion/entry inhibitors. A formulation of the anti-HIV vaginal microbicide RC1 analog RC-101 has been developed (Sassi *et al.*, 2011a,b). A polyvinyl alcohol vaginal membrane containing RC-101 (100 μg/membrane) was shown to be safe in human and monkey tissue and effective against HIV-1 *in vitro* and *ex vivo* in human cervical tissue. RC-101 films are stable for 1 month at 25°C and maintain *in vitro* bioactivity for up to 6 months (Sassi *et al.*, 2011b). Therefore, RC-101 has the potential to be a safe and effective topical microbicide.

Human papilloma virus

The main routes of human papilloma virus (HPV) transmission are sexual transmission, contact transmission and mother-to-child transmission. According to their oncogenic potential, HPV can be divided into low-risk and high-risk types (LR-HPV and HR-HPV) (Tommasino, 2014; Doorbar *et al.*, 2015). Infection with different types of HPV can cause a variety of diseases, such as genital warts and cervical cancer. The α-defensins resist HPV infection mainly through immune regulation, preventing HPV from entering target cells and altering HPV transport in cells. HNP1 can directly promote the apoptosis of condyloma acuminatum cells and exert an antiviral effect in condyloma acuminatum tissue by restricting viral replication (Zhao *et al.*, 2015). HNP2 can recruit DCs in tumor lesions caused by HPV infection (Hubert *et al.*, 2007). HD5 is particularly effective against sexually transmitted HPV types (Buck *et al.*, 2006) by stabilizing the viral capsid and completely blocking endosome uncoating via consolidating the interaction between the two capsid proteins (L1/L2) of HPV (Gulati *et al.*, 2019). HD5 has at least two effects on HPV entry: firstly, blocking the cleavage of the minor capsid protein L2 on the cell surface (Wiens and Smith, 2015); and secondly, even though the virus is internalized and partially uncoated, the genome remains abnormally associated with the major L1 capsid protein and cannot enter the nucleus (Wiens and Smith, 2017). After HPV entry into cells, HD5 treatment significantly altered the trafficking of the viral genome and capsid proteins downstream of the early endosome, redirecting them from the trans-Golgi network to the lysosome. Hence capsid proteins are degraded more rapidly during infection in the presence of HD5 (Wiens and Smith, 2017). HPV infection can induce the expression of defensins, mainly including HBD2 and HBD3, in relevant lesions (Kreuter *et al.*, 2009; Erhart *et al.*, 2011; Mhatre *et al.*, 2012; Buckley *et al.*, 2016; Dasgupta *et al.*, 2016; Szukiewicz *et al.*, 2016). β-defensin gene polymorphisms and CNV are associated with HPV susceptibility (Abe *et al.*, 2013; Segat *et al.*, 2014). However, the relevant mechanisms and therapeutic potential of β-defensins against HPV infection still

need to be studied and evaluated. θ-defensins inhibit high-risk HPV infection through charge-driven capsid clustering (Skeate et al., 2020).

Candidiasis

Candida yeast invades the vagina and cause mucosal inflammation called vulvovaginal candidiasis. The vaginal epithelial cells provide the first barrier against fungi, actively recognize the pathogenic phenotype of fungi, and initiate local responses by releasing antimicrobial peptides, chemokines and other cytokines (Netea and Kullberg, 2010; Yano et al., 2010). Local innate immunity plays a key role in the defence and pathogenesis of vaginal *Candida* infection.

Vaginas infected by *C. albicans* have high numbers of polysexual neutrophils (PMNs). PMN-derived proteins can cause macrophages to engulf bacteria. Human neutrophil peptides (HNP1–3) secreted by the PMN are mediators of macrophage activation (Soehnlein et al., 2008). *In vitro* studies have shown that HNP1 and HNP2 exhibit potent *Candida* killing activity, whereas HNP3 is completely inactive against *C. albicans* due to an asparagine residue at its N-terminus (Raj et al., 2000). HD6 prevents adhesion, invasion and biofilm formation of the fungal pathogen (Cegelski, 2017; Chairatana et al., 2017).

Candida infection induces the expression and production of β-defensins, which are also lethal to the fungi (Pivarcsi et al., 2005; Kotani et al., 2020; Miró et al., 2021). Selective stimulation of the amnion chorion by *C. albicans* results in tissue-specific secretion of HBD1 and HBD2 (Zaga-Clavellina et al., 2012b). HBD2 and HBD3 have potent killing activity against *Candida* at micromolar concentrations, with HBD3 being ten times more powerful than HBD2 (Joly et al., 2004; Vylkova et al., 2006). *In vitro* experiments have shown that HBD2 kills *C. albicans* through membrane permeability mediated by phosphatidylinositol 4,5-bisphosphate (Järvå et al., 2018). In addition, Vylkova et al. (2007) proved that HBD2 and HBD3 kill *Candida* in an energy-dependent and salt-sensitive manner without disrupting the membrane. Kotani et al. (2020) found that *C. albicans* induced HBD2 expression in a human vaginal epithelial cell line, VK2/E6E7, by activating Dectin-1 and (NF-κB) signaling. In animal experiments, increased fungal burden and mucosal invasion trigger local transcriptional levels of mBD1 in TLR2−/− mice (Miró et al., 2017).

Defensins in human reproduction

Defensins at the maternal–fetal interface

The maternal–fetal interface promotes tolerance to the semiexogeneic fetuses while also preventing vertical transmission of infection. As an indirect 'dialogue' contact, the maternal–fetal interface is constituted by different components at different stages of pregnancy: first, the endometrium during the 'implantation window' and the trophoblasts in the implantation stage, and later, the basal decidua and villous chorion, which is called placenta (Ander et al., 2019). Different defensin levels have been reported in the above interfaces. During the implantation window, HNP1–3 are expressed by endometrial stromal cells (Das et al., 2007). HBD1–4 are expressed in the endometrium with varied peaks throughout the menstrual cycle (as discussed above). However, none of them seem to participate in the implantation process other than the antimicrobial function (King et al., 2003b; Das et al., 2007). Existing studies have proven that HNP1–3 proteins are expressed in amniotic fluid (Table I) (Espinoza et al., 2003), placental trophoblasts (Svinarich et al., 1997) and vernix caseosa (Akinbi et al., 2004). HD5 mRNA is expressed in chorion (Svinarich et al., 1997). The HBD1–3 mRNAs and proteins are detected in the trophoblasts of the placenta and chorion, amnion epithelium, amniotic fluid and decidua in term and preterm pregnancy (Buhimschi et al., 2004; King et al., 2007a,b). Concentrations of HBD1 in amniotic fluid vary with gestational age and are higher in the second trimester (Varrey et al., 2018). The fetal foreskin only expresses HBD2 among the β-defensins at mRNA levels (Dorschner et al., 2003).

Under the circumstances of pregnancy disorders, abnormal levels of defensins levels were found to be accompanied with specific situations. Microbial invasion of the amniotic membrane, premature delivery and premature rupture of premature fetal membranes are related to the increased concentration of amniotic fluid immunoreactive HNP1–3 (Espinoza et al., 2003). Elevated vaginal fluid neutrophil defensins (HNP1–3) concentrations at 24–29 weeks gestation may predict preterm birth before 32 weeks (Balu et al., 2003). Human beta defensin-1 protein levels show no significant differences between asymptomatic women with a history of spontaneous preterm birth or cervical surgery, both of whom have a high risk of preterm birth (Manning et al., 2019). The concentration of HBD2 in the amniotic fluid in the second trimester was associated with premature rupture of membranes but not with preterm birth (Iavazzo et al., 2010). A recent study on the relationship between vaginal microbiota and spontaneous preterm birth (sPTB) in US women reported that higher vaginal levels of HBD2 lowered the risk of sPTB associated with cervicovaginal microbiota in African American women, but not in non-African American women, independently of the presence or absence of *Lactobacillus* species, which are the major species in vaginal microbiota. However, the mechanism behind this observation is still unknown (Elovitz et al., 2019). As a physiological component in the amniotic fluid, HBD3 increases during the process of termed labor and also increases in women with spontaneous preterm labor with intact membranes or preterm prelabor rupture of membranes with intra-amniotic inflammation or intra-amniotic infection. In addition, according to the proteomics of amniotic fluid, four biomarkers (defensin-1, defensin-2, calgranulin C and calgranulin A) that are highly predictive of intrauterine inflammation have been shown to detect inflammation accurately, through the Mr score. The Mr score shows the gradient of disease activity, from 'absent' to 'mild' to 'severe' inflammation (Buhimschi et al., 2006). This method provides an opportunity for the early identification of chorioamnionitis (Buhimschi et al., 2008), which can identify patients who may benefit from uterine intervention in the modern diagnosis-treatment framework. Other studies also show that defensins play a certain role in the antimicrobial infection and inflammation of the mother-fetal interface. Tissue-specific HBD1–3, secreted by the outer cell membrane of the human placenta, is stimulated by *E. coli* (Garcia-Lopez et al., 2010). Stimulation with *G. vaginalis* induces a tissue-specific secretion profile of HBD1–3 in the chorioamniotic membranes (Zaga-Clavellina et al., 2012a). Additionally, in patients undergoing assisted reproduction, the expression level of HBD1 in the follicular fluid of women with a good fertilization rate is significantly higher than that in women with poor fertilization rates (Zupin et al., 2019a).

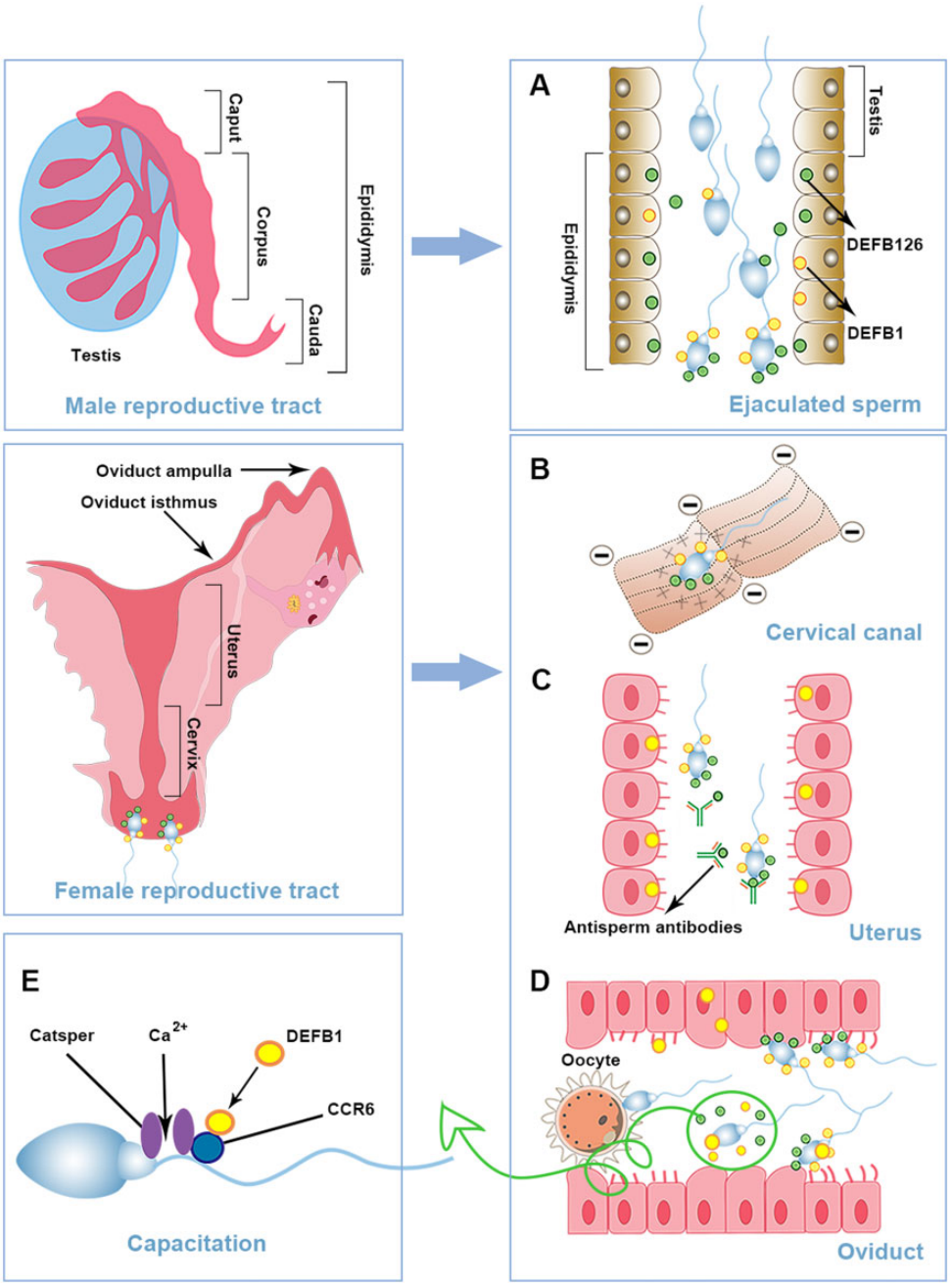


Figure 5. The role of defensins in human reproduction. Defensins are widely distributed along the male and female reproductive tracts. Sperm are produced from the testis, mature in the epididymis and enter the female reproductive tract for fertilization. (**A**) During the maturation in the epididymis, β-defensins are loaded onto the sperm surface. (**B**) Defensins are cationic polypeptides, while cervical mucus proteins are negatively charged. Therefore, defensins can promote sperm to pass through the cervical mucus to the uterus. (**C**) DEFB126 can also form a protective shield on the surface of sperm to protect the sperm from immune recognition and binding by anti-sperm antibodies. (**D**) When the sperm reaches the isthmus of the fallopian tube, the DEFB126 on the surface of the sperm can mediate the combination of the sperm with the fallopian tube epithelium to form a sperm pool. When sperm capacitate, the surface DEFB126 falls off, the sperm is released from the isthmus of the fallopian tube, and the receptor of the sperm and oocyte on the surface are exposed to promote fertilization. (**E**) DEFB1 secreted from the uterine epithelium interacts with sperm chemokine receptor type 6 (CCR6) and causes Catsper-dependent Ca^{2+} influx to maintain movement.

The role of defensins in male fertility

During storage in the epididymis, the sperm undergoes a series of profound changes in morphological structure, biochemical metabolism and physiological functions, and finally obtains the capacity for movement and sperm–oocyte binding. In animal experiments, defensins in the epididymis affect the maturation, transport, capacitation and fertilization of sperm (Yudin *et al.*, 2005a; Tollner *et al.*, 2008a; Fernandez-Fuertes *et al.*, 2016; Lyons *et al.*, 2018). In some species, certain β-defensin clusters are preferentially expressed in the MRT and are involved in reproduction (Patil *et al.*, 2005; Zhou *et al.*, 2013). However, due to the difficulties in obtaining human epididymal epithelium or alternative *in vitro* cellular models, the majority of research on β-defensin function in male fertility has been done in animals.

Beta-defensins expressed in the epididymis play a major role in male infertility due to their involvement in sperm maturation, transportation and capacitation (Zhou *et al.*, 2004a; Dorin and Barratt, 2014; Zhang *et al.*, 2018). When sperm passes through the epididymal duct, defensins are adsorbed to the entire sperm surface (Yudin *et al.*, 2005b, 2008; Tollner *et al.*, 2008b) (Fig. 5A). DEFB126 prevents the female immune system from recognizing sperm, hence, it is essential for the effective transport of sperm in the FRT in macaque (Yudin *et al.*, 2005a); it also mediates attachment of sperm to oviductal epithelia in macaque and bull (Tollner *et al.*, 2008a; Lyons *et al.*, 2018) and promotes the delivery of active sperm to the fertilization site in macaque (Yudin *et al.*, 2005b) (Fig. 5B–D). Mouse DEFB41 affects the combination of sperm and zona pellucida (Björkgren *et al.*, 2016).

Due to ethical reasons, research on defensin in human sperm maturation has not yet been reported. However, related clinical studies have found that β-defensins on human sperm correlate with sperm motility, bactericidal effects and fertilization rates. The loss of DEFB126 is related to male infertility. The proportion of DEFB126-positive sperm in normal semen samples was significantly higher than in patients with varicocele and infertile semen deficiency (Aram *et al.*, 2020). A form of *DEFB126* mutation, double nucleotide deletion (*DEFB126*-2del) or tetranucleotide deletion (*DEFB126*-4del), is associated with decreased sperm motility (Ozerlat, 2011; Tollner *et al.*, 2011), and full-length *DEFB126* can increase sperm motility *in vitro* (Aram *et al.*, 2020). The allele frequency of the double nucleotide deletion (*DEFB126*-2del) variant sequence is very high in the European (0.47) and Chinese (0.45) population cohorts. Homozygous patients for this mutation are not sterile, but their fertilization rate is extremely low (Tollner *et al.*, 2011). Moreover, homozygous patients with this mutation among infertility patients were significantly higher than those in the control group ($P \leq 0.05$), and the existence of this deletion led to a significant reduction in the clinical pregnancy rate of intrauterine fertilization ($P \leq 0.05$) (Boroujeni *et al.*, 2019). In addition, the sperm penetration rate of *DEFB126*-2del patients through a hyaluronic acid gel was 84% lower than that of other genotypes, mainly due to the changes in the glycocalyx on the sperm surface (Tollner *et al.*, 2011). Lectin chip technology analyzed the difference between mutant *DEFB126* and wild-type sperm surface glycocalyx and finally found that six types of lectins (Jacalin/AIA, GHA, ACAL, MPL, MPL, VVL, ABA) and homozygous *DEFB126* mutant spermatozoa have a lower binding affinity (Xin *et al.*, 2016). In summary, DEFB126, especially its glycosylation, plays a vital role in the sperm function.

Besides its antimicrobial function, HBD1 was found to relate to sperm function. Diao *et al.* (2014) showed that compared with normal fertile sperm, the content of HBD1 in the sperm of infertile men with leukospermia or asthenospermia is much lower. Interference in HBD function leads to reduced motility and bactericidal activity of normal sperm. Recombinant HBD1 treatment could significantly restore HBD1 expression, bactericidal activity, sperm quality and the oocyte penetration ability in the sperm of patients with asthenospermia and leukospermia (Diao *et al.*, 2014). Consistent with the above finding, Zupin *et al.* (2019a) found that the concentrations of HBD1 are lower in oligo-asthenozoospermic semen than in normozoospermic semen, and the incubation of exogenous exposure HBD1 significantly increased normal sperm motility after 1 h and 24 h. However, the results of Khayamabed *et al.* are different from those of Zupin *et al.* Their results showed that incubating sperm with HBD1 maintained the percentage of sperm motility within 2 h ($P < 0.05$). In contrast, after 3 h, the difference was reduced and insignificant (Khayamabed *et al.*, 2020). The differences between these studies may be related to the source, stability of recombinant HBD1 and experimental conditions (room temperature vs 37°C), as well as the method of sperm preparation. The underlying mechanism of HBD1 affecting various sperm functions is that it is a ligand of chemokine receptor type 6 (CCR6), which mediates the ligand-induced, CatSper-dependent Ca^{2+} influx required for these functions (Diao *et al.*, 2014; 2017). On the other hand, in the FRT, HBD1 is reported to be secreted by the cumulus–oocyte complex and the uterine epithelium in response to elevated progesterone levels around the ovulation period (Calogero *et al.*, 2000; Horne *et al.*, 2008; Yin *et al.*, 2009). Therefore, after sperm enters FRT, HBD1 of female origin also promotes sperm motility and helps them to reach the fallopian tube.

Conclusion

At present, antibiotic resistance is an important issue in treating reproductive tract infections (Unemo and Jensen, 2017). Macrolide antibiotics and fluoroquinolones are used to treat common mycoplasma infections in the genital tract, and related mutations that are resistant to these two drugs cause treatment failure (Li *et al.*, 2020; Machalek *et al.*, 2020; Gossé *et al.*, 2021). In some low-income and middle-income countries, the increase in drug-resistant strains of AIDS is mainly due to poor antiretroviral therapy, incomplete adherence to treatment and poor viral load testing (Hamers *et al.*, 2018). Therefore, there is an urgent need to adopt global surveillance and measures to optimize the effects of reproductive tract infection treatment, including drug resistance-guided strategies and new treatment methods to ensure that a high proportion of reproductive tract infections are treated successfully. As a broad-spectrum antimicrobial peptides without drug resistance, defensins have great potential in developing a new natural antimicrobial therapy to treat reproductive tract infections (Wilmes and Sahl, 2014; Suarez-Carmona *et al.*, 2015). However, problems remain in developing and translating defensins as anti-infective therapies and fertility-improving drugs. Defensins suffer from potential toxicity, poor stability, low bioavailability and difficult synthesis. Especially with HIV infection, defensins are potentially pathogenic in special biological environments, and their safety needs to be carefully evaluated. The development and innovation of defensin drugs can provide new ideas for

the clinical treatment of reproductive tract infections and related infertility problems.

Data availability

The data underlying this article are available in GEO database, and can be accessed with GeneChip array data GSE7307 and high-throughput sequencing data GSE150852.

Authors' roles

Y.-J.Z. and Y.F. conceived the manuscript, participated in writing, prepared the tables and the figures and reviewed the content of the work. X.M. and F.M. conceived the manuscript, participated in the article revision and reviewed the content of the work. All authors reviewed the final text and agreed to take responsibility for all work.

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Conflict of interest

The authors declare that there are no conflicts of interest.

References

Abe S, Miura K, Kinoshita A, Mishima H, Miura S, Yamasaki K, Hasegawa Y, Higashijima A, Jo O, Sasaki K *et al*. Copy number variation of the antimicrobial-gene, defensin beta 4, is associated with susceptibility to cervical cancer. *J Hum Genet* 2013;**58**: 250–253.

Agerberth B, Charo J, Werr J, Olsson B, Idali F, Lindbom L, Kiessling R, Jörnvall H, Wigzell H, Gudmundsson GH. The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. *Blood* 2000;**96**:3086–3093.

Aguilar-Jiménez W, Zapata W, Rugeles MT. Differential expression of human beta defensins in placenta and detection of allelic variants in the DEFB1 gene from HIV-1 positive mothers. *Biomedica* 2011; **31**:44–54.

Akinbi HT, Narendran V, Pass AK, Markart P, Hoath SB. Host defense proteins in vernix caseosa and amniotic fluid. *Am J Obstet Gynecol* 2004;**191**:2090–2096.

Ander SE, Diamond MS, Coyne CB. Immune responses at the maternal-fetal interface. *Sci Immunol* 2019;**4**:eaat6114.

Anderson J, Akkina R. HIV-1 resistance conferred by siRNA cosuppression of CXCR4 and CCR5 coreceptors by a bispecific lentiviral vector. *AIDS Res Ther* 2005;**2**:1.

Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. *J Pathol* 2008;**214**:231–241.

Aram R, Chan PTK, Cyr DG. Beta-defensin126 is correlated with sperm motility in fertile and infertile men. *Biol Reprod* 2020;**102**: 92–101.

Balu RB, Savitz DA, Ananth CV, Hartmann KE, Miller WC, Thorp JM, Heine RP. Bacterial vaginosis and vaginal fluid defensins during pregnancy. *Am J Obstet Gynecol* 2002;**187**:1267–1271.

Balu RB, Savitz DA, Ananth CV, Hartmann KE, Miller WC, Thorp JM, Heine RP. Bacterial vaginosis, vaginal fluid neutrophil defensins, and preterm birth. *Obstet Gynecol* 2003;**101**:862–868.

Baroncelli S, Ricci E, Andreotti M, Guidotti G, Germano P, Marazzi MC, Vella S, Palombi L, De Rossi A, Giuliano M. Single-nucleotide polymorphisms in human beta-defensin-1 gene in Mozambican HIV-1-infected women and correlation with virologic parameters. *AIDS* 2008;**22**:1515–1517.

Barratt CL, Bolton AE, Cooke ID. Functional significance of white blood cells in the male and female reproductive tract. *Hum Reprod* 1990;**5**:639–648.

Becknell B, Spencer JD, Carpenter AR, Chen X, Singh A, Ploeger S, Kline J, Ellsworth P, Li B, Proksch E *et al*. Expression and antimicrobial function of beta-defensin 1 in the lower urinary tract. *PLoS One* 2013;**8**:e77714.

Bensch KW, Raida M, Mägert HJ, Schulz-Knappe P, Forssmann WG. hBD-1: a novel beta-defensin from human plasma. *FEBS Lett* 1995; **368**:331–335.

Bevins CL, Jones DE, Dutra A, Schaffzin J, Muenke M. Human enteric defensin genes: chromosomal map position and a model for possible evolutionary relationships. *Genomics* 1996;**31**:95–106.

Bharucha JP, Sun L, Lu W, Gartner S, Garzino-Demo A. Human beta-defensin 2 and 3 inhibit HIV-1 replication in macrophages. *Front Cell Infect Microbiol* 2021;**11**:535352.

Biswas B, Bhushan S, Rajesh A, Suraj SK, Lu Y, Meinhardt A, Yenugu S. Uropathogenic *Escherichia coli* (UPEC) induced antimicrobial gene expression in the male reproductive tract of rat: evaluation of the potential of Defensin 21 to limit infection. *Andrology* 2015;**3**: 368–375.

Biswas B, Yenugu S. Antimicrobial responses in the male reproductive tract of lipopolysaccharide challenged rats. *Am J Reprod Immunol* 2011;**65**:557–568.

Biswas B, Yenugu S. Lipopolysaccharide induces epididymal and testicular antimicrobial gene expression *in vitro*: insights into the epigenetic regulation of sperm-associated antigen 11e gene. *Immunogenetics* 2013;**65**:239–253.

Biswas B, Yenugu S. Transcriptional regulation of the rat sperm-associated antigen 11e (Spag 11e) gene during endotoxin challenge. *Mol Genet Genomics* 2014;**289**:837–845.

Björkgren I, Alvarez L, Blank N, Balbach M, Turunen H, Laajala TD, Toivanen J, Krutskikh A, Wahlberg N, Huhtaniemi I *et al*. Targeted inactivation of the mouse epididymal beta-defensin 41 alters sperm flagellar beat pattern and zona pellucida binding. *Mol Cell Endocrinol* 2016;**427**:143–154.

Boroujeni PB, Ebrahimian S, Abedini M, Chayjan MR, Hassani M, Gourabi H, Sadighi-Gilani MA, Sabbaghian M, Meybodi AM. The role of DEFB126 variation in male infertility and medically assisted reproduction technique outcome. *Reprod Biomed Online* 2019;**39**: 649–657.

Brunham RC, Paavonen J. Reproductive system infections in women: lower genital tract syndromes. *Pathog Dis* 2020;**78**:ftaa022.

Brunner A, Medvecz M, Makra N, Sárdy M, Komka K, Gugolya M, Szabó D, Gajdács M, Ostorházi E. Human beta defensin levels and vaginal microbiome composition in post-menopausal women diagnosed with lichen sclerosus. *Sci Rep* 2021;**11**:15999.

Buck CB, Day PM, Thompson CD, Lubkowski J, Lu W, Lowy DR, Schiller JT. Human alpha-defensins block papillomavirus infection. *Proc Natl Acad Sci USA* 2006;**103**:1516–1521.

Buckley N, Huber A, Lo Y, Castle PE, Kemal K, Burk RD, Strickler HD, Einstein MH, Young M, Anastos K *et al.* Association of high-risk human papillomavirus with genital tract mucosal immune factors in HIV-infected women. *Am J Reprod Immunol* 2016;**75**: 146–154.

Buhimschi CS, Weiner CP, Buhimschi IA. Proteomics, part II: the emerging role of proteomics over genomics in spontaneous preterm labor/birth. *Obstet Gynecol Surv* 2006;**61**:543–553.

Buhimschi IA, Jabr M, Buhimschi CS, Petkova AP, Weiner CP, Saed GM. The novel antimicrobial peptide beta3-defensin is produced by the amnion: a possible role of the fetal membranes in innate immunity of the amniotic cavity. *Am J Obstet Gynecol* 2004;**191**: 1678–1687.

Buhimschi IA, Zambrano E, Pettker CM, Bahtiyar MO, Paidas M, Rosenberg VA, Thung S, Salafia CM, Buhimschi CS. Using proteomic analysis of the human amniotic fluid to identify histologic chorioamnionitis. *Obstet Gynecol* 2008;**111**:403–412.

Burris HH, Riis VM, Schmidt I, Gerson KD, Brown A, Elovitz MA. Maternal stress, low cervicovaginal β-defensin, and spontaneous preterm birth. *Am J Obstet Gynecol MFM* 2020;**2**:100092.

Calogero AE, Burrello N, Barone N, Palermo I, Grasso U, D'agata R. Effects of progesterone on sperm function: mechanisms of action. *Hum Reprod* 2000;**15**(Suppl 1):28–45.

Cao D, Li Y, Yang R, Wang Y, Zhou Y, Diao H, Zhao Y, Zhang Y, Lu J. Lipopolysaccharide-induced epididymitis disrupts epididymal beta-defensin expression and inhibits sperm motility in rats. *Biol Reprod* 2010;**83**:1064–1070.

Castro J, Jefferson KK, Cerca N. Innate immune components affect growth and virulence traits of bacterial-vaginosis-associated and non-bacterial-vaginosis-associated *Gardnerella vaginalis* strains similarly. *Pathog Dis* 2018;**76**:10.

Cegelski L. Disentangling nanonets: human α-defensin 6 targets *Candida albicans* virulence. *Biochemistry* 2017;**56**:1027–1028.

Chairatana P, Chiang IL, Nolan EM. Human α-defensin 6 self-assembly prevents adhesion and suppresses virulence traits of *Candida albicans*. *Biochemistry* 2017;**56**:1033–1041.

Chang TL, Vargas J, Delportillo A, Klotman ME. Dual role of alpha-defensin-1 in anti-HIV-1 innate immunity. *J Clin Invest* 2005;**115**: 765–773.

Chen F, Tang Y, Zheng H, Xu Y, Wang J, Wang C. Roles of the conserved amino acid residues in reduced human defensin 5: cysteine and arginine are indispensable for its antibacterial action and LPS neutralization. *ChemMedChem* 2019;**14**:1457–1465.

Chertov O, Michiel DF, Xu L, Wang JM, Tani K, Murphy WJ, Longo DL, Taub DD, Oppenheim JJ. Identification of defensin-1, defensin-2, and CAP37/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils. *J Biol Chem* 1996; **271**:2935–2940.

Cole AL, Yang OO, Warren AD, Waring AJ, Lehrer RI, Cole AM. HIV-1 adapts to a retrocyclin with cationic amino acid substitutions that reduce fusion efficiency of gp41. *J Immunol* 2006;**176**: 6900–6905.

Cole AM, Hong T, Boo LM, Nguyen T, Zhao C, Bristol G, Zack JA, Waring AJ, Yang OO, Lehrer RI. Retrocyclin: a primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1. *Proc Natl Acad Sci USA* 2002;**99**:1813–1818.

Com E, Bourgeon F, Evrard B, Ganz T, Colleu D, Jégou B, Pineau C. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. *Biol Reprod* 2003;**68**:95–104.

Conejo-Garcia JR, Benencia F, Courreges M-C, Kang E, Mohamed-Hadley A, Buckanovich RJ, Holtz DO, Jenkins A, Na H, Zhang L et al Tumor-infiltrating dendritic cell precursors recruited by a beta-defensin contribute to vasculogenesis under the influence of Vegf-A. *Nat Med* 2004;**10**:950–958.

Das S, Bates MD, Vince GS, Lewis-Jones I, Gazvani R. Follicular fluid expression of alpha-defensins and their role in ovulation. *J Assist Reprod Genet* 2008;**25**:83–87.

Das S, Vince GS, Lewis-Jones I, Bates MD, Gazvani R. The expression of human alpha and beta defensin in the endometrium and their effect on implantation. *J Assist Reprod Genet* 2007;**24**: 533–539.

Dasgupta T, Nweze EI, Yue H, Wang L, Jin J, Ghosh SK, Kawsar HI, Zender C, Androphy EJ, Weinberg A *et al.* Human papillomavirus oncogenic E6 protein regulates human β-defensin 3 (hBD3) expression via the tumor suppressor protein p53. *Oncotarget* 2016;**7**: 27430–27444.

De Leeuw E, Li C, Zeng P, Li C, Diepeveen-De Buin M, Lu WY, Breukink E, Lu W. Functional interaction of human neutrophil peptide-1 with the cell wall precursor lipid II. *FEBS Lett* 2010;**584**: 1543–1548.

Demirkhanyan LH, Marin M, Padilla-Parra S, Zhan C, Miyauchi K, Jean-Baptiste M, Novitskiy G, Lu W, Melikyan GB. Multifaceted mechanisms of HIV-1 entry inhibition by human α-defensin. *J Biol Chem* 2012;**287**:28821–28838.

Diao H, Yu H-G, Sun F, Zhang Y-L, Tanphaichitr N. Rat recombinant β-defensin 22 is a heparin-binding protein with antimicrobial activity. *Asian J Androl* 2011;**13**:305–311.

Diao R, Fok KL, Chen H, Yu MK, Duan Y, Chung CM, Li Z, Wu H, Li Z, Zhang H *et al.* Deficient human β-defensin 1 underlies male infertility associated with poor sperm motility and genital tract infection. *Sci Transl Med* 2014;**6**:249ra108.

Diao R, Wang T, Fok KL, Li X, Ruan Y, Yu MK, Cheng Y, Chen Y, Chen H, Mou L *et al.* CCR6 is required for ligand-induced CatSper activation in human sperm. *Oncotarget* 2017;**8**:91445–91458.

Ding J, Tasker C, Valere K, Sihvonen T, Descalzi-Montoya DB, Lu W, Chang TL. Anti-HIV activity of human defensin 5 in primary CD4+ T cells under serum-deprived conditions is a consequence of defensin-mediated cytotoxicity. *PLoS One* 2013;**8**:e76038.

Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. *Rev Med Virol* 2015;**25**(Suppl 1):2–23.

Dorin JR, Barratt CLR. Importance of β-defensins in sperm function. *Mol Hum Reprod* 2014;**20**:821–826.

Dorschner RA, Lin KH, Murakami M, Gallo RL. Neonatal skin in mice and humans expresses increased levels of antimicrobial

peptides: innate immunity during development of the adaptive response. *Pediatr Res* 2003;**53**:566–572.

Driss V, Legrand F, Hermann E, Loiseau S, Guerardel Y, Kremer L, Adam E, Woerly G, Dombrowicz D, Capron M. TLR2-dependent eosinophil interactions with mycobacteria: role of alpha-defensins. *Blood* 2009;**113**:3235–3244.

Dubé E, Hermo L, Chan PTK, Cyr DG. Alterations in gene expression in the caput epididymides of nonobstructive azoospermic men. *Biol Reprod* 2008;**78**:342–351.

Eade CR, Diaz C, Wood MP, Anastos K, Patterson BK, Gupta P, Cole AL, Cole AM. Identification and characterization of bacterial vaginosis-associated pathogens using a comprehensive cervical-vaginal epithelial coculture assay. *PLoS One* 2012;**7**:e50106.

Ellegård R, Shankar EM, Larsson M. Targeting HIV-1 innate immune responses therapeutically. *Curr Opin HIV AIDS* 2011;**6**:435–443.

Elovitz MA, Gajer P, Riis V, Brown AG, Humphrys MS, Holm JB, Ravel J. Cervicovaginal microbiota and local immune response modulate the risk of spontaneous preterm delivery. *Nat Commun* 2019;**10**:1305.

Erhart W, Alkasi Ö, Brunke G, Wegener F, Maass N, Arnold N, Arlt A, Meinhold-Heerlein I. Induction of human β-defensins and psoriasin in vulvovaginal human papillomavirus-associated lesions. *J Infect Dis* 2011;**204**:391–399.

Espinoza J, Chaiworapongsa T, Romero R, Edwin S, Rathnasabapathy C, Gomez R, Bujold E, Camacho N, Kim YM, Hassan S *et al.* Antimicrobial peptides in amniotic fluid: defensins, calprotectin and bacterial/permeability-increasing protein in patients with microbial invasion of the amniotic cavity, intra-amniotic inflammation, preterm labor and premature rupture of membranes. *J Matern Fetal Neonatal Med* 2003;**13**:2–21.

Fan SR, Liu XP, Liao QP. Human defensins and cytokines in vaginal lavage fluid of women with bacterial vaginosis. *Int J Gynaecol Obstet* 2008;**103**:50–54.

Fei Z, Hu S, Xiao L, Zhou J, Diao H, Yu H, Fang S, Wang Y, Wan Y, Wang W *et al.* mBin1b transgenic mice show enhanced resistance to epididymal infection by bacteria challenge. *Genes Immun* 2012; **13**:445–451.

Feng Z, Dubyak GR, Jia X, Lubkowski JT, Weinberg A. Human β-defensin-3 structure motifs that are important in CXCR4 antagonism. *FEBS J* 2013;**280**:3365–3375.

Feng Z, Dubyak GR, Lederman MM, Weinberg A. Cutting edge: human beta defensin 3—a novel antagonist of the HIV-1 coreceptor CXCR4. *J Immunol* 2006;**177**:782–786.

Fernandez-Fuertes B, Narciandi F, O'farrelly C, Kelly AK, Fair S, Meade KG, Lonergan P. Cauda epididymis-specific beta-defensin 126 promotes sperm motility but not fertilizing ability in cattle. *Biol Reprod* 2016;**95**:122.

Fichorova RN, Chen P-L, Morrison CS, Doncel GF, Mendonca K, Kwok C, Chipato T, Salata R, Mauck C. The contribution of cervicovaginal infections to the immunomodulatory effects of hormonal contraception. *mBio* 2015;**6**:e00221–e00215.

Fichorova RN, Morrison CS, Chen P-L, Yamamoto HS, Govender Y, Junaid D, Ryan S, Kwok C, Chipato T, Salata RA *et al.* Aberrant cervical innate immunity predicts onset of dysbiosis and sexually transmitted infections in women of reproductive age. *PLoS One* 2020;**15**:e0224359.

Fichorova RN, Rheinwald JG, Anderson DJ. Generation of papillomavirus-immortalized cell lines from normal human ectocervical, endocervical, and vaginal epithelium that maintain expression of tissue-specific differentiation proteins. *Biol Reprod* 1997;**57**: 847–855.

Fleming DC, King AE, Williams ARW, Critchley HOD, Kelly RW. Hormonal contraception can suppress natural antimicrobial gene transcription in human endometrium. *Fertil Steril* 2003;**79**:856–863.

Flores-Espinosa P, Pineda-Torres M, Vega-Sánchez R, Estrada-Gutiérrez G, Espejel-Nuñez A, Flores-Pliego A, Maida-Claros R, Paredes-Vivas Y, Morales-Méndez I, Sosa-González I *et al.* Progesterone elicits an inhibitory effect upon LPS-induced innate immune response in pre-labor human amniotic epithelium. *Am J Reprod Immunol* 2014;**71**:61–72.

Fruitwala S, El-Naccache DW, Chang TL. Multifaceted immune functions of human defensins and underlying mechanisms. *Semin Cell Dev Biol* 2019;**88**:163–172.

Furci L, Sironi F, Tolazzi M, Vassena L, Lusso P. Alpha-defensins block the early steps of HIV-1 infection: interference with the binding of gp120 to CD4. *Blood* 2007;**109**:2928–2935.

Furci L, Tolazzi M, Sironi F, Vassena L, Lusso P. Inhibition of HIV-1 infection by human α-defensin-5, a natural antimicrobial peptide expressed in the genital and intestinal mucosae. *PLoS One* 2012;**7**: e45208.

Gabay JE, Scott RW, Campanelli D, Griffith J, Wilde C, Marra MN, Seeger M, Nathan CF. Antibiotic proteins of human polymorphonuclear leukocytes. *Proc Natl Acad Sci USA* 1989;**86**:5610–5614.

Gallo SA, Wang W, Rawat SS, Jung G, Waring AJ, Cole AM, Lu H, Yan X, Daly NL, Craik DJ *et al.* Theta-defensins prevent HIV-1 Env-mediated fusion by binding gp41 and blocking 6-helix bundle formation. *J Biol Chem* 2006;**281**:18787–18792.

Ganz T. Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol* 2003;**3**:710–720.

Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI. Defensins. Natural peptide antibiotics of human neutrophils. *J Clin Invest* 1985;**76**:1427–1435.

Garcia AE, Osapay G, Tran PA, Yuan J, Selsted ME. Isolation, synthesis, and antimicrobial activities of naturally occurring theta-defensin isoforms from baboon leukocytes. *Infect Immun* 2008;**76**: 5883–5891.

Garcia-Lopez G, Flores-Espinosa P, Zaga-Clavellina V. Tissue-specific human beta-defensins (HBD)1, HBD2, and HBD3 secretion from human extra-placental membranes stimulated with *Escherichia coli*. *Reprod Biol Endocrinol* 2010;**8**:146–146.

Ghartey JP, Carpenter C, Gialanella P, Rising C, Mcandrew TC, Mhatre M, Tugetman J, Einstein MH, Chazotte C, Herold BC. Association of bactericidal activity of genital tract secretions with *Escherichia coli* colonization in pregnancy. *Am J Obstet Gynecol* 2012;**207**:297.e1–297.e8.

Ghosh M, Jais M, Delisle J, Younes N, Benyeogor I, Biswas R, Mohamed H, Daniels J, Wang C, Young M *et al.* Dysregulation in genital tract soluble immune mediators in postmenopausal women is distinct by HIV status. *AIDS Res Hum Retroviruses* 2019;**35**: 251–259.

Ghosh M, Schaefer TM, Fahey JV, Wright JA, Wira CR. Antiviral responses of human Fallopian tube epithelial cells to toll-like receptor 3 agonist poly(I:C). *Fertil Steril* 2008;**89**:1497–1506.

Gossé M, Nordbø SA, Pukstad B. Evaluation of treatment with two weeks of doxycycline on macrolide-resistant strains of *Mycoplasma genitalium*: a retrospective observational study. *BMC Infect Dis* 2021;**21**:1225.

Gulati NM, Miyagi M, Wiens ME, Smith JG, Stewart PL. α-defensin HD5 stabilizes capsid/core interactions. *Pathog Immun* 2019;**4**: 196–234.

Guthrie BL, Introini A, Roxby AC, Choi RY, Bosire R, Lohman-Payne B, Hirbod T, Farquhar C, Broliden K. Depot medroxyprogesterone acetate use is associated with elevated innate immune effector molecules in cervicovaginal secretions of HIV-1-uninfected women. *J Acquir Immune Defic Syndr* 2015;**69**:1–10.

Hall SH, Yenugu S, Radhakrishnan Y, Avellar MCW, Petrusz P, French FS. Characterization and functions of beta defensins in the epididymis. *Asian J Androl* 2007;**9**:453–462.

Hamers RL, Rinke De Wit TF, Holmes CB. HIV drug resistance in low-income and middle-income countries. *Lancet HIV* 2018;**5**: e588–e596.

Harder J, Bartels J, Christophers E, Schroder JM. A peptide antibiotic from human skin. *Nature* 1997;**387**:861.

Harder J, Bartels J, Christophers E, Schroder JM. Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. *J Biol Chem* 2001;**276**:5707–5713.

Hardwick RJ, Amogne W, Mugusi S, Yimer G, Ngaimisi E, Habtewold A, Minzi O, Makonnen E, Janabi M, Machado LR *et al.* β-defensin genomic copy number is associated with HIV load and immune reconstitution in sub-saharan Africans. *J Infect Dis* 2012; **206**:1012–1019.

Hayashi R, Tsuchiya K, Fukushima K, Horita N, Hibiya S, Kitagaki K, Negi M, Itoh E, Akashi T, Eishi Y *et al.* Reduced human alpha-defensin 6 in noninflamed jejunal tissue of patients with Crohn's disease. *Inflamm Bowel Dis* 2016;**22**:1119–1128.

Hein M, Valore EV, Helmig RB, Uldbjerg N, Ganz T. Antimicrobial factors in the cervical mucus plug. *Am J Obstet Gynecol* 2002;**187**: 137–144.

Hirbod T, Kong X, Kigozi G, Ndyanabo A, Serwadda D, Prodger JL, Tobian AA, Nalugoda F, Wawer MJ, Shahabi K *et al.* HIV acquisition is associated with increased antimicrobial peptides and reduced HIV neutralizing IgA in the foreskin prepuce of uncircumcised men. *PLoS Pathog* 2014;**10**:e1004416.

Holly MK, Diaz K, Smith JG. Defensins in viral infection and pathogenesis. *Annu Rev Virol* 2017;**4**:369–391.

Hong SA, Kim KH, Lee TJ, Park ES, Kim MK, Myung SC. A role of human beta defensin-1 in predicting prostatic adenocarcinoma in cases of false-negative biopsy. *APMIS* 2017;**125**:1063–1069.

Horne AW, Stock SJ, King AE. Innate immunity and disorders of the female reproductive tract. *Reproduction* 2008;**135**:739–749.

Hou J, Liu H-Y, Diao H, Yu H. The truncated human beta-defensin 118 can modulate lipopolysaccharide mediated inflammatory response in RAW264.7 macrophages. *Peptides* 2021;**136**:170438.

Hu H, Di B, Tolbert WD, Gohain N, Yuan W, Gao P, Ma B, He Q, Pazgier M, Zhao L *et al.* Systematic mutational analysis of human neutrophil α-defensin HNP4. *Biochim Biophys Acta Biomembr* 2019; **1861**:835–844.

Hu S, Yao G, Guan X, Ni Z, Ma W, Wilson EM, French FS, Liu Q, Zhang Y. Research resource: genome-wide mapping of *in vivo* androgen receptor binding sites in mouse epididymis. *Mol Endocrinol* 2010;**24**:2392–2405.

Hu S-G, Zou M, Yao G-X, Ma W-B, Zhu Q-L, Li X-Q, Chen Z-J, Sun Y. Androgenic regulation of beta-defensins in the mouse epididymis. *Reprod Biol Endocrinol* 2014;**12**:76–76.

Hubert P, Herman L, Maillard C, Caberg J-H, Nikkels A, Pierard G, Foidart J-M, Noel A, Boniver J, Delvenne P. Defensins induce the recruitment of dendritic cells in cervical human papillomavirus-associated (pre)neoplastic lesions formed *in vitro* and transplanted *in vivo*. *FASEB J* 2007;**21**:2765–2775.

Hubert P, Herman L, Roncarati P, Maillard C, Renoux V, Demoulin S, Erpicum C, Foidart J-M, Boniver J, Noël A *et al.* Altered α-defensin 5 expression in cervical squamocolumnar junction: implication in the formation of a viral/tumour-permissive microenvironment. *J Pathol* 2014;**234**:464–477.

Hughes BL, Dutt R, Raker C, Barthelemy M, Rossoll RM, Ramratnam B, Wira CR, Cu-Uvin S. The impact of pregnancy on anti-HIV activity of cervicovaginal secretions. *Am J Obstet Gynecol* 2016;**215**: 748.e1–748.e12.

Iavazzo C, Tassis K, Gourgiotis D, Boutsikou M, Baka S, Hassiakos D, Hadjithomas A, Botsis D, Malamitsi-Puchner A. The role of human beta defensins 2 and 3 in the second trimester amniotic fluid in predicting preterm labor and premature rupture of membranes. *Arch Gynecol Obstet* 2010;**281**:793–799.

Iwasaki A. Antiviral immune responses in the genital tract: clues for vaccines. *Nat Rev Immunol* 2010;**10**:699–711.

Jais M, Younes N, Chapman S, Cu-Uvin S, Ghosh M. Reduced levels of genital tract immune biomarkers in postmenopausal women: implications for HIV acquisition. *Am J Obstet Gynecol* 2016;**215**: 324.e1–324.e10.

Jalkanen J, Huhtaniemi I, Poutanen M. Discovery and characterization of new epididymis-specific beta-defensins in mice. *Biochim Biophys Acta* 2005;**1730**:22–30.

Järvå M, Phan TK, Lay FT, Caria S, Kvansakul M, Hulett MD. Human β-defensin 2 kills through phosphatidylinositol 4,5-bisphosphate-mediated membrane permeabilization. *Sci Adv* 2018;**4**:eaat0979.

Jiang W, Ghosh SK, Flyckt R, Kalinowska M, Starks D, Jurevic R, Weinberg A, Lederman MM, Rodriguez B. Bacterial colonization and beta defensins in the female genital tract in HIV infection. *Curr HIV Res* 2012;**10**:504–512.

Jin G, Weinberg A. Human antimicrobial peptides and cancer. *Semin Cell Dev Biol* 2019;**88**:156–162.

John M, Keller MJ, Fam EH, Cheshenko N, Hogarty K, Kasowitz A, Wallenstein S, Carlucci MJ, Tuyama AC, Lu W *et al.* Cervicovaginal secretions contribute to innate resistance to herpes simplex virus infection. *J Infect Dis* 2005;**192**:1731–1740.

Joly S, Maze C, Mccray PB, Guthmiller JM. Human beta-defensins 2 and 3 demonstrate strain-selective activity against oral microorganisms. *J Clin Microbiol* 2004;**42**:1024–1029.

Jones DE, Bevins CL. Defensin-6 mRNA in human Paneth cells: implications for antimicrobia peptides in host defense of the human bowel. *FEBS Lett* 1993;**315**:187–192.

Jones DE, Bevins CL. Paneth cells of the human small intestine express an antimicrobial peptide gene. *J Biol Chem* 1992;**267**: 23216–23225.

Keller MJ, Huber A, Espinoza L, Serrano MG, Parikh HI, Buck GA, Gold JA, Wu Y, Wang T, Herold BC. Impact of herpes simplex

virus type 2 and human immunodeficiency virus dual infection on female genital tract mucosal immunity and the vaginal microbiome. *J Infect Dis* 2019;**220**:852–861.

Khayamabed R, Tavalaee M, Taherian S-S, Nasr-Esfahani MH. Effect of recombinant β-defensin 1 protein on human sperm motility and viability. *Andrologia* 2020;**52**:e13455.

Kim C, Gajendran N, Mittrucker HW, Weiwad M, Song YH, Hurwitz R, Wilmanns M, Fischer G, Kaufmann SH. Human alpha-defensins neutralize anthrax lethal toxin and protect against its fatal consequences. *Proc Natl Acad Sci USA* 2005;**102**: 4830–4835.

Kim JH, Kim K-H, Kim HJ, Lee J, Myung SC. Expression of beta-defensin 131 promotes an innate immune response in human prostate epithelial cells. *PLoS One* 2015;**10**:e0144776.

Kim K-H, Lee J, Han JH, Myung SC. Beta-defensin 124 is required for efficient innate immune responses in prostate epithelial RWPE-1 cells. *Korean J Urol* 2014;**55**:417–425.

King AE, Critchley HOD, Kelly RW. Innate immune defences in the human endometrium. *Reprod Biol Endocrinol* 2003a;**1**:116.

King AE, Fleming DC, Critchley HO, Kelly RW. Differential expression of the natural antimicrobials, beta-defensins 3 and 4, in human endometrium. *J Reprod Immunol* 2003b;**59**:1–16.

King AE, Fleming DC, Critchley HOD, Kelly RW. Regulation of natural antibiotic expression by inflammatory mediators and mimics of infection in human endometrial epithelial cells. *Mol Hum Reprod* 2002;**8**:341–349.

King AE, Kelly RW, Sallenave JM, Bocking AD, Challis JRG. Innate immune defences in the human uterus during pregnancy. *Placenta* 2007a;**28**:1099–1106.

King AE, Paltoo A, Kelly RW, Sallenave JM, Bocking AD, Challis JRG. Expression of natural antimicrobials by human placenta and fetal membranes. *Placenta* 2007b;**28**:161–169.

Klotman ME, Chang TL. Defensins in innate antiviral immunity. *Nat Rev Immunol* 2006;**6**:447–456.

Klotman ME, Rapista A, Teleshova N, Micsenyi A, Jarvis GA, Lu W, Porter E, Chang TL. *Neisseria gonorrhoeae*-induced human defensins 5 and 6 increase HIV infectivity: role in enhanced transmission. *J Immunol* 2008;**180**:6176–6185.

Kohli M, Young CY, Tindall DJ, Nandy D, Mckenzie KM, Bevan GH, Donkena KV. Whole blood defensin mRNA expression is a predictive biomarker of docetaxel response in castration-resistant prostate cancer. *Onco Targets Ther* 2015;**8**:1915–1922.

Kotani H, Koshizuka T, Matsubara K, Nishiyama K, Sugiyama T, Suzutani T. Relationship between human β-defensin 2 and the vaginal environment. *Jpn J Infect Dis* 2020;**73**:214–220.

Kreuter A, Skrygan M, Gambichler T, Brockmeyer NH, Stücker M, Herzler C, Potthoff A, Altmeyer P, Pfister H, Wieland U. Human papillomavirus-associated induction of human beta-defensins in anal intraepithelial neoplasia. *Br J Dermatol* 2009;**160**:1197–1205.

Lafferty MK, Sun L, Christensen-Quick A, Lu W, Garzino-Demo A. Human beta defensin 2 selectively inhibits HIV-1 in highly permissive $CCR6^{+}CD4^{+}$ T cells. *Viruses* 2017;**9**:111.

Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends Immunol* 2009;**30**: 131–141.

Lehrer RI. Primate defensins. *Nat Rev Microbiol* 2004;**2**:727–738.

Lehrer RI, Barton A, Daher KA, Harwig SS, Ganz T, Selsted ME. Interaction of human defensins with *Escherichia coli*. Mechanism of bactericidal activity. *J Clin Invest* 1989;**84**:553–561.

Lehrer RI, Cole AM, Selsted ME. θ-Defensins: cyclic peptides with endless potential. *J Biol Chem* 2012;**287**:27014–27019.

Lehrer RI, Ganz T. Defensins of vertebrate animals. *Curr Opin Immunol* 2002;**14**:96–102.

Lehrer RI, Lu W. α-Defensins in human innate immunity. *Immunol Rev* 2012;**245**:84–112.

Levinson P, Choi RY, Cole AL, Hirbod T, Rhedin S, Payne B, Guthrie BL, Bosire R, Cole AM, Farquhar C *et al.* HIV-neutralizing activity of cationic polypeptides in cervicovaginal secretions of women in HIV-serodiscordant relationships. *PLoS One* 2012;**7**:e31996.

Levinson P, Kaul R, Kimani J, Ngugi E, Moses S, Macdonald KS, Broliden K, Hirbod T; Kibera HIV Study Group. Levels of innate immune factors in genital fluids: association of alpha defensins and LL-37 with genital infections and increased HIV acquisition. *AIDS* 2009;**23**:309–317.

Li P, Chan HC, He B, So SC, Chung YW, Shang Q, Zhang YD, Zhang YL. An antimicrobial peptide gene found in the male reproductive system of rats. *Science* 2001;**291**:1783–1785.

Li WN, Shi L, Long XY, Li Y, Zhu WB, Liu G. *Mycoplasma genitalium* incidence, treatment failure, and resistance: a retrospective survey of men of infertile couples from a hospital in China. *Andrology* 2020;**8**:91–100.

Libby EK, Pascal KE, Mordechai E, Adelson ME, Trama JP. Atopobium vaginae triggers an innate immune response in an *in vitro* model of bacterial vaginosis. *Microbes Infect* 2008;**10**: 439–446.

Lin Q, Xie K, Chen D, Yu B, Mao X, Yu J, Luo J, Zheng P, Luo Y, Yan H *et al.* Expression and functional characterization of a novel antimicrobial peptide: human beta-defensin 118. *Biomed Res Int* 2020;**2020**:1395304.

Lin YQ, Li JY, Wang HY, Liu J, Zhang CL, Wang WT, Liu J, Li N, Jin SH. Cloning and identification of a novel sperm binding protein, HEL-75, with antibacterial activity and expressed in the human epididymis. *Hum Reprod* 2008;**23**:2086–2094.

Linzmeier R, Michaelson D, Liu L, Ganz T. The structure of neutrophil defensin genes. *FEBS Lett* 1993;**326**:299–300.

Liu H, Yu H, Gu Y, Xin A, Zhang Y, Diao H, Lin D. Human beta-defensin DEFB126 is capable of inhibiting LPS-mediated inflammation. *Appl Microbiol Biotechnol* 2013;**97**:3395–3408.

Liu L, Zhao C, Heng HH, Ganz T. The human beta-defensin-1 and alpha-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. *Genomics* 1997;**43**:316–320.

Lucovnik M, Kornhauser-Cerar L, Premru-Srsen T, Gmeiner-Stopar T, Derganc M. Neutrophil defensins but not interleukin-6 in vaginal fluid after preterm premature rupture of membranes predict fetal/neonatal inflammation and infant neurological impairment. *Acta Obstet Gynecol Scand* 2011;**90**:908–916.

Lyons A, Narciandi F, Donnellan E, Romero-Aguirregomezcorta J, Farrelly CO, Lonergan P, Meade KG, Fair S. Recombinant β-defensin 126 promotes bull sperm binding to bovine oviductal epithelia. *Reprod Fertil Dev* 2018;**30**:1472–1481.

Machalek DA, Tao Y, Shilling H, Jensen JS, Unemo M, Murray G, Chow EPF, Low N, Garland SM, Vodstrcil LA *et al.* Prevalence of

mutations associated with resistance to macrolides and fluoroquinolones in *Mycoplasma genitalium*: a systematic review and meta-analysis. *Lancet Infect Dis* 2020;**20**:1302–1314.

Manning R, James CP, Smith MC, Innes BA, Stamp E, Peebles D, Bajaj-Elliott M, Klein N, Bulmer JN, Robson SC *et al*. Predictive value of cervical cytokine, antimicrobial and microflora levels for pre-term birth in high-risk women. *Sci Rep* 2019;**9**:11246.

Mars WM, Patmasiriwat P, Maity T, Huff V, Weil MM, Saunders GF. Inheritance of unequal numbers of the genes encoding the human neutrophil defensins HP-1 and HP-3. *J Biol Chem* 1995;**270**: 30371–30376.

Mawhinney M, Mariotti A. Physiology, pathology and pharmacology of the male reproductive system. *Periodontol 2000* 2013;**61**: 232–251.

Mcconaghy JR, Panchal B. Epididymitis: an overview. *Am Fam Physician* 2016;**94**:723–726.

Mehlotra RK, Dazard J-E, John B, Zimmerman PA, Weinberg A, Jurevic RJ. Copy number variation within human β-defensin gene cluster influences progression to AIDS in the multicenter AIDS cohort study. *J AIDS Clin Res* 2012;**3**:1000184.

Mendling W. Vaginal microbiota. *Adv Exp Med Biol* 2016;**902**:83–93.

Meng W, Du R, Wang Y, Chen Z, Ding Y. Human β-defensin messenger RNA is overexpressed in the cervical epithelia of patients with nongonococcal cervicitis. *J Low Genit Tract Dis* 2013;**17**: 440–445.

Mhatre M, Mcandrew T, Carpenter C, Burk RD, Einstein MH, Herold BC. Cervical intraepithelial neoplasia is associated with genital tract mucosal inflammation. *Sex Transm Dis* 2012;**39**:591–597.

Milanese M, Segat L, Arraes LC, Garzino-Demo A, Crovella S. Copy number variation of defensin genes and HIV infection in Brazilian children. *J Acquir Immune Defic Syndr* 2009;**50**:331–333.

Milanese M, Segat L, Pontillo A, Arraes LC, De Lima Filho JL, Crovella S. DEFB1 gene polymorphisms and increased risk of HIV-1 infection in Brazilian children. *AIDS* 2006;**20**:1673–1675.

Miró MS, Caeiro JP, Rodriguez E, Vargas L, Vigezzi C, Icely PA, Castillo GDV, Azcurra AI, Abiega CD, Riera FO *et al*. *Candida albicans* modulates murine and human beta defensin-1 during vaginitis. *J Fungi (Basel)* 2021;**8**:20.

Miró MS, Rodríguez E, Vigezzi C, Icely PA, García LN, Peinetti N, Maldonado CA, Riera FO, Caeiro JP, Sotomayor CE. Contribution of TLR2 pathway in the pathogenesis of vulvovaginal candidiasis. *Pathog Dis* 2017;**75**:10.

Mitchell C, Gottsch ML, Liu C, Fredricks DN, Nelson DB. Associations between vaginal bacteria and levels of vaginal defensins in pregnant women. *Am J Obstet Gynecol* 2013;**208**: 132.e1–132.e137.

Miyai K, Yonekura Y, Ito K, Matsukuma S, Tsuda H. Gene expression microarray analysis of adult testicular germ cell tumor: a comparison between pure-type seminomas and seminoma components in mixed tumors. *Virchows Arch* 2021;**479**:1177–1186.

Moazzezy N, Asadi Karam MR, Rafati S, Bouzari S, Oloomi M. Inhibition and eradication activity of truncated α-defensin analogs against multidrug resistant uropathogenic *Escherichia coli* biofilm. *PLoS One* 2020;**15**:e0235892.

Monin L, Whettlock EM, Male V. Immune responses in the human female reproductive tract. *Immunology* 2020;**160**:106–115.

Mukura LR, Hickey DK, Rodriguez-Garcia M, Fahey JV, Wira CR. *Chlamydia trachomatis* regulates innate immune barrier integrity and mediates cytokine and antimicrobial responses in human uterine ECC-1 epithelial cells. *Am J Reprod Immunol* 2017;**78**:e12764.

Münk C, Wei G, Yang OO, Waring AJ, Wang W, Hong T, Lehrer RI, Landau NR, Cole AM. The theta-defensin, retrocyclin, inhibits HIV-1 entry. *AIDS Res Hum Retroviruses* 2003;**19**:875–881.

Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* 2006;**6**:173–182.

Netea MG, Kullberg BJ. Epithelial sensing of fungal invasion. *Cell Host Microbe* 2010;**8**:219–220.

Niyonsaba F, Ogawa H, Nagaoka I. Human beta-defensin-2 functions as a chemotactic agent for tumour necrosis factor-alpha-treated human neutrophils. *Immunology* 2004;**111**:273–281.

Noda-Nicolau NM, Bastos LB, Bolpetti AN, Pinto GVS, Marcolino LD, Marconi C, Ferreira CST, Polettini J, Vieira EP, Da Silva MG. Cervicovaginal levels of human β-defensin 1, 2, 3, and 4 of reproductive-aged women with *Chlamydia trachomatis* infection. *J Low Genit Tract Dis* 2017;**21**:189–192.

Noda-Nicolau NM, Silva MDC, Bento GFC, Ferreira JSB, Novak J, Morales J, Tronco JA, Bolpetti AN, Pinto GVS, Polettini J *et al*. Cervicovaginal levels of human beta defensins during bacterial vaginosis. *PLoS One* 2021;**16**:e0260753.

Nold C, Maubert M, Anton L, Yellon S, Elovitz MA. Prevention of preterm birth by progestational agents: what are the molecular mechanisms? *Am J Obstet Gynecol* 2013;**208**:223.e1–223.e7.

Oeckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF-κB signaling pathways. *Nat Immunol* 2011;**12**:695–708.

Oh J, Lee J, Woo J-M, Choi E, Park I, Han C, Baek N, Lee H, Kim DH, Cho C. Systematic identification and integrative analysis of novel genes expressed specifically or predominantly in mouse epididymis. *BMC Genomics* 2006;**7**:314.

O'Neil DA. Regulation of expression of beta-defensins: endogenous enteric peptide antibiotics. *Mol Immunol* 2003;**40**:445–450.

Ordonez SR, Veldhuizen EJA, Van Eijk M, Haagsman HP. Role of soluble innate effector molecules in pulmonary defense against fungal pathogens. *Front Microbiol* 2017;**8**:2098.

Ouellette AJ. Paneth cell α-defensins in enteric innate immunity. *Cell Mol Life Sci* 2011;**68**:2215–2229.

Ozerlat I. Male factor infertility: mutation of sperm defensin causes subfertility. *Nat Rev Urol* 2011;**8**:474–474.

Page-Shafer K, Sweet S, Kassaye S, Ssali C. (C2) Saliva, breast milk, and mucosal fluids in HIV transmission. *Adv Dent Res* 2006;**19**: 152–157.

Palladino MA, Mallonga TA, Mishra MS. Messenger RNA (mRNA) expression for the antimicrobial peptides beta-defensin-1 and beta-defensin-2 in the male rat reproductive tract: beta-defensin-1 mRNA in initial segment and caput epididymidis is regulated by androgens and not bacterial lipopolysaccharides. *Biol Reprod* 2003; **68**:509–515.

Para R, Romero R, Miller D, Panaitescu B, Varrey A, Chaiworapongsa T, Hassan SS, Hsu C-D, Gomez-Lopez N. Human β-defensin-3 participates in intra-amniotic host defense in women with labor at term, spontaneous preterm labor and intact membranes, and preterm prelabor rupture of membranes. *J Matern Fetal Neonatal Med* 2020;**33**:4117–4132.

Patil AA, Cai Y, Sang Y, Blecha F, Zhang G. Cross-species analysis of the mammalian beta-defensin gene family: presence of syntenic gene clusters and preferential expression in the male reproductive tract. *Physiol Genomics* 2005;**23**:5–17.

Patrão MTCC, Silva EJR, Avellar MCW. Androgens and the male reproductive tract: an overview of classical roles and current perspectives. *Arq Bras Endocrinol Metabol* 2009;**53**:934–945.

Pazgier M, Hoover DM, Yang D, Lu W, Lubkowski J. Human beta-defensins. *Cell Mol Life Sci* 2006;**63**:1294–1313.

Pazgier M, Li X, Lu W, Lubkowski J. Human defensins: synthesis and structural properties. *Curr Pharm Des* 2007;**13**:3096–3118.

Pellett Madan R, Masson L, Tugetman J, Werner L, Grobler A, Mlisana K, Lo Y, Che D, Arnold KB, Abdool Karim SS *et al*. Innate antibacterial activity in female genital tract secretions is associated with increased risk of HIV acquisition. *AIDS Res Hum Retroviruses* 2015;**31**:1153–1159.

Penberthy WT, Chari S, Cole AL, Cole AM. Retrocyclins and their activity against HIV-1. *Cell Mol Life Sci* 2011;**68**:2231–2242.

Petrov V, Funderburg N, Weinberg A, Sieg S. Human β defensin-3 induces chemokines from monocytes and macrophages: diminished activity in cells from HIV-infected persons. *Immunology* 2013;**140**: 413–420.

Pivarcsi A, Nagy I, Koreck A, Kis K, Kenderessy-Szabo A, Szell M, Dobozy A, Kemeny L. Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. *Microbes Infect* 2005;**7**: 1117–1127.

Polettini J, Takitane J, Peraçoli JC, Silva MG. Expression of β defensins 1, 3 and 4 in chorioamniotic membranes of preterm pregnancies complicated by chorioamnionitis. *Eur J Obstet Gynecol Reprod Biol* 2011;**157**:150–155.

Pope M, Haase AT. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. *Nat Med* 2003;**9**: 847–852.

Porter E, Yang H, Yavagal S, Preza GC, Murillo O, Lima H, Greene S, Mahoozi L, Klein-Patel M, Diamond G *et al*. Distinct defensin profiles in *Neisseria gonorrhoeae* and *Chlamydia trachomatis* urethritis reveal novel epithelial cell-neutrophil interactions. *Infect Immun* 2005;**73**:4823–4833.

Premratanachai P, Joly S, Johnson GK, Mccray PB, Jia HP, Guthmiller JM. Expression and regulation of novel human beta-defensins in gingival keratinocytes. *Oral Microbiol Immunol* 2004;**19**:111–117.

Prodger JL, Hirbod T, Kigozi G, Nalugoda F, Reynolds SJ, Galiwango R, Shahabi K, Serwadda D, Wawer MJ, Gray RH *et al*.; Rakai Genital Immunology Research Group. Immune correlates of HIV exposure without infection in foreskins of men from Rakai, Uganda. *Mucosal Immunol* 2014;**7**:634–644.

Pujianto DA, Loanda E, Sari P, Midoen YH, Soeharso P. Sperm-associated antigen 11A is expressed exclusively in the principal cells of the mouse caput epididymis in an androgen-dependent manner. *Reprod Biol Endocrinol* 2013;**11**:59.

Pujianto DA, Muliawati D, Rizki MD, Parisudha A, Hardiyanto L. Mouse defensin beta 20 (Defb20) is expressed specifically in the caput region of the epididymis and regulated by androgen and testicular factors. *Reprod Biol* 2020;**20**:536–540.

Quayle AJ, Porter EM, Nussbaum AA, Wang YM, Brabec C, Yip KP, Mok SC. Gene expression, immunolocalization, and secretion of human defensin-5 in human female reproductive tract. *Am J Pathol* 1998;**152**:1247–1258.

Radhakrishnan Y, Hamil KG, Yenugu S, Young SL, French FS, Hall SH. Identification, characterization, and evolution of a primate beta-defensin gene cluster. *Genes Immun* 2005;**6**:203–210.

Raj PA, Antonyraj KJ, Karunakaran T. Large-scale synthesis and functional elements for the antimicrobial activity of defensins. *Biochem J* 2000;**347** Pt 3:633–641.

Rapista A, Ding J, Benito B, Lo Y-T, Neiditch MB, Lu W, Chang TL. Human defensins 5 and 6 enhance HIV-1 infectivity through promoting HIV attachment. *Retrovirology* 2011;**8**:45–45.

Ricci E, Malacrida S, Zanchetta M, Montagna M, Giaquinto C, De Rossi A. Role of beta-defensin-1 polymorphisms in mother-to-child transmission of HIV-1. *J Acquir Immune Defic Syndr* 2009;**51**:13–19.

Robertson MJ, Kent K, Tharp N, Nozawa K, Dean L, Mathew M, Grimm SL, Yu Z, Légaré C, Fujihara Y *et al*. Large-scale discovery of male reproductive tract-specific genes through analysis of RNA-seq datasets. *BMC Biol* 2020;**18**:103.

Rodríguez-García M, Oliva H, Climent N, García F, Gatell JM, Gallart T. Human immature monocyte-derived dendritic cells produce and secrete alpha-defensins 1-3. *J Leukoc Biol* 2007;**82**:1143–1146.

Rodríguez-Jiménez F-J, Krause A, Schulz S, Forssmann W-G, Conejo-Garcia J-R, Schreeb R, Motzkus D. Distribution of new human β-defensin genes clustered on chromosome 20 in functionally different segments of epididymis. *Genomics* 2003;**81**:175–183.

Sang Y, Ortega MT, Blecha F, Prakash O, Melgarejo T. Molecular cloning and characterization of three beta-defensins from canine testes. *Infect Immun* 2005;**73**:2611–2620.

Sangeeta K, Yenugu S. Male reproductive tract antimicrobial expression in the extremes of ages of rats. *Gene* 2019;**710**:218–232.

Sasani N, Roghanian R, Emtiazi G, Aghaie A. A novel approach on leukodepletion filters: investigation of synergistic anticancer effect of purified α-defensins and nisin. *Adv Pharm Bull* 2021;**11**:378–384.

Sassi AB, Bunge KE, Hood BL, Conrads TP, Cole AM, Gupta P, Rohan LC. Preformulation and stability in biological fluids of the retrocyclin RC-101, a potential anti-HIV topical microbicide. *AIDS Res Ther* 2011a;**8**:27.

Sassi AB, Cost MR, Cole AL, Cole AM, Patton DL, Gupta P, Rohan LC. Formulation development of retrocyclin 1 analog RC-101 as an anti-HIV vaginal microbicide product. *Antimicrob Agents Chemother* 2011b;**55**:2282–2289.

Sawada M, Otsuki K, Mitsukawa K, Yakuwa K, Nagatsuka M, Okai T. Cervical inflammatory cytokines and other markers in the cervical mucus of pregnant women with lower genital tract infection. *Int J Gynaecol Obstet* 2006;**92**:117–121.

Schutte BC, Mccray PB. [beta]-defensins in lung host defense. *Annu Rev Physiol* 2002;**64**:709–748.

Schutte BC, Mitros JP, Bartlett JA, Walters JD, Jia HP, Welsh MJ, Casavant TL, Mccray PB. Discovery of five conserved beta-defensin gene clusters using a computational search strategy. *Proc Natl Acad Sci USA* 2002;**99**:2129–2133.

Segat L, Brandão LA, Guimarães RL, Crovella S. Are defensin beta 1 gene polymorphisms associated with HIV infection and virus replication? *AIDS* 2009;**23**:647–650.

Segat L, Zupin L, Moura RR, Coelho AVC, Chagas BS, De Freitas AC, Crovella S. DEFB1 polymorphisms are involved in

susceptibility to human papillomavirus infection in Brazilian gynaecological patients. *Mem Inst Oswaldo Cruz* 2014;**109**:918–922.

Seidel A, Ye Y, De Armas LR, Soto M, Yarosh W, Marcsisin RA, Tran D, Selsted ME, Camerini D. Cyclic and acyclic defensins inhibit human immunodeficiency virus type-1 replication by different mechanisms. *PLoS One* 2010;**5**:e9737.

Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. *Nat Immunol* 2005;**6**:551–557.

Selsted ME, Tang YQ, Morris WL, Mcguire PA, Novotny MJ, Smith W, Henschen AH, Cullor JS. Purification, primary structures, and antibacterial activities of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. *J Biol Chem* 1993;**268**:6641–6648.

Semple CAM, Rolfe M, Dorin JR. Duplication and selection in the evolution of primate beta-defensin genes. *Genome Biol* 2003;**4**:R31.

Semple F, Dorin JR. beta-Defensins: multifunctional modulators of infection, inflammation and more? *J Innate Immun* 2012;**4**:337–348.

Sfanos KS, Wilson BA, De Marzo AM, Isaacs WB. Acute inflammatory proteins constitute the organic matrix of prostatic corpora amylacea and calculi in men with prostate cancer. *Proc Natl Acad Sci USA* 2009;**106**:3443–3448.

Shafee TMA, Lay FT, Phan TK, Anderson MA, Hulett MD. Convergent evolution of defensin sequence, structure and function. *Cell Mol Life Sci* 2017;**74**:663–682.

Sharma R, Gupta S, Agarwal A, Henkel R, Finelli R, Parekh N, Saleh R, Arafa M, Ko E, Zini A *et al.* Relevance of leukocytospermia and semen culture and its true place in diagnosing and treating male infertility. *World J Mens Health* 2022;**40**:191–207.

Shust GF, Cho S, Kim M, Madan RP, Guzman EM, Pollack M, Epstein J, Cohen HW, Keller MJ, Herold BC. Female genital tract secretions inhibit herpes simplex virus infection: correlation with soluble mucosal immune mediators and impact of hormonal contraception. *Am J Reprod Immunol* 2010;**63**:110–119.

Silva EJR, Ribeiro CM, Mirim AFM, Silva AAS, Romano RM, Hallak J, Avellar MCW. Lipopolysaccharide and lipotheicoic acid differentially modulate epididymal cytokine and chemokine profiles and sperm parameters in experimental acute epididymitis. *Sci Rep* 2018;**8**:103.

Skeate JG, Segerink WH, Garcia MD, Fernandez DJ, Prins R, Lühen KP, Voss FO, Da Silva DM, Kast WM. Theta-defensins inhibit high-risk human papillomavirus infection through charge-driven capsid clustering. *Front Immunol* 2020;**11**:561843.

Soehnlein O, Kai-Larsen Y, Frithiof R, Sorensen OE, Kenne E, Scharffetter-Kochanek K, Eriksson EE, Herwald H, Agerberth B, Lindbom L. Neutrophil primary granule proteins HBP and HNP1-3 boost bacterial phagocytosis by human and murine macrophages. *J Clin Invest* 2008;**118**:3491–3502.

Soto E, Espinoza J, Nien JK, Kusanovic JP, Erez O, Richani K, Santolaya-Forgas J, Romero R. Human beta-defensin-2: a natural antimicrobial peptide present in amniotic fluid participates in the host response to microbial invasion of the amniotic cavity. *J Matern Fetal Neonatal Med* 2007;**20**:15–22.

Sparkes RS, Kronenberg M, Heinzmann C, Daher KA, Klisak I, Ganz T, Mohandas T. Assignment of defensin gene(s) to human chromosome 8p23. *Genomics* 1989;**5**:240–244.

Spencer JD, Hains DS, Porter E, Bevins CL, Dirosario J, Becknell B, Wang H, Schwaderer AL. Human alpha defensin 5 expression in the human kidney and urinary tract. *PLoS One* 2012;**7**:e31712.

Suarez-Carmona M, Hubert P, Delvenne P, Herfs M. Defensins: "Simple" antimicrobial peptides or broad-spectrum molecules? *Cytokine Growth Factor Rev* 2015;**26**:361–370.

Suff N, Karda R, Diaz JA, Ng J, Baruteau J, Perocheau D, Taylor PW, Alber D, Buckley SMK, Bajaj-Elliott M *et al.* Cervical gene delivery of the antimicrobial peptide, human β-defensin (HBD)-3, in a mouse model of ascending infection-related preterm birth. *Front Immunol* 2020;**11**:106.

Svinarich DM, Gomez R, Romero R. Detection of human defensins in the placenta. *Am J Reprod Immunol* 1997;**38**:252–255.

Szukiewicz D, Alkhalayla H, Pyzlak M, Watroba M, Szewczyk G, Wejman J. Human beta-defensin 1, 2 and 3 production by amniotic epithelial cells with respect to human papillomavirus (HPV) infection, HPV oncogenic potential and the mode of delivery. *Microb Pathog* 2016;**97**:154–165.

Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010;**140**:805–820.

Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunol Rev* 2012;**249**:158–175.

Tang YQ, Yuan J, Osapay G, Osapay K, Tran D, Miller CJ, Ouellette AJ, Selsted ME. A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins. *Science* 1999;**286**:498–502.

Tantengco OAG, Kechichian T, Vincent KL, Pyles RB, Medina PMB, Menon R. Inflammatory response elicited by *Ureaplasma parvum* colonization in human cervical epithelial, stromal, and immune cells. *Reproduction* 2021;**163**:1–10.

Territo MC, Ganz T, Selsted ME, Lehrer R. Monocyte-chemotactic activity of defensins from human neutrophils. *J Clin Invest* 1989;**84**:2017–2020.

Tollner TL, Bevins CL, Cherr GN. Multifunctional glycoprotein DEFB126–a curious story of defensin-clad spermatozoa. *Nat Rev Urol* 2012;**9**:365–375.

Tollner TL, Venners SA, Hollox EJ, Yudin AI, Liu X, Tang G, Xing H, Kays RJ, Lau T, Overstreet JW *et al.* A common mutation in the defensin DEFB126 causes impaired sperm function and subfertility. *Sci Transl Med* 2011;**3**:92ra65.

Tollner TL, Yudin AI, Tarantal AF, Treece CA, Overstreet JW, Cherr GN. Beta-defensin 126 on the surface of macaque sperm mediates attachment of sperm to oviductal epithelia. *Biol Reprod* 2008a;**78**:400–412.

Tollner TL, Yudin AI, Treece CA, Overstreet JW, Cherr GN. Macaque sperm coating protein DEFB126 facilitates sperm penetration of cervical mucus. *Hum Reprod* 2008b;**23**:2523–2534.

Tommasino M. The human papillomavirus family and its role in carcinogenesis. *Semin Cancer Biol* 2014;**26**:13–21.

Unemo M, Jensen JS. Antimicrobial-resistant sexually transmitted infections: gonorrhoea and *Mycoplasma genitalium*. *Nat Rev Urol* 2017;**14**:139–152.

Valere K, Lu W, Chang TL. Key determinants of human α-defensin 5 and 6 for enhancement of HIV infectivity. *Viruses* 2017;**9**:244.

Valere K, Rapista A, Eugenin E, Lu W, Chang TL. Human alpha-defensin HNP1 increases HIV traversal of the epithelial barrier: a potential role in STI-mediated enhancement of HIV transmission. *Viral Immunol* 2015;**28**:609–615.

Valore EV, Park CH, Quayle AJ, Wiles KR, Mccray PB, Ganz T. Human beta-defensin-1: an antimicrobial peptide of urogenital tissues. *J Clin Invest* 1998;**101**:1633–1642.

Valore EV, Wiley DJ, Ganz T. Reversible deficiency of antimicrobial polypeptides in bacterial vaginosis. *Infect Immun* 2006;**74**: 5693–5702.

Varrey A, Romero R, Panaitescu B, Miller D, Chaiworapongsa T, Patwardhan M, Faro J, Pacora P, Hassan SS, Hsu C-D *et al*. Human β-defensin-1: a natural antimicrobial peptide present in amniotic fluid that is increased in spontaneous preterm labor with intra-amniotic infection. *Am J Reprod Immunol* 2018;**80**:e13031.

Verbanac D, Zanetti M, Romeo D. Chemotactic and protease-inhibiting activities of antibiotic peptide precursors. *FEBS Lett* 1993; **317**:255–258.

Vivier E, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, Mckenzie ANJ, Mebius RE *et al*. Innate lymphoid cells: 10 years on. *Cell* 2018;**174**:1054–1066.

Vylkova S, Li XS, Berner JC, Edgerton M. Distinct antifungal mechanisms: beta-defensins require *Candida albicans* Ssa1 protein, while Trk1p mediates activity of cysteine-free cationic peptides. *Antimicrob Agents Chemother* 2006;**50**:324–331.

Vylkova S, Nayyar N, Li W, Edgerton M. Human beta-defensins kill *Candida albicans* in an energy-dependent and salt-sensitive manner without causing membrane disruption. *Antimicrob Agents Chemother* 2007;**51**:154–161.

Wang W, Cole AM, Hong T, Waring AJ, Lehrer RI. Retrocyclin, an antiretroviral theta-defensin, is a lectin. *J Immunol* 2003;**170**: 4708–4716.

Wang W, Owen SM, Rudolph DL, Cole AM, Hong T, Waring AJ, Lal RB, Lehrer RI. Activity of alpha- and theta-defensins against primary isolates of HIV-1. *J Immunol* 2004;**173**:515–520.

Weber A, Alves J, Abujamra AL, Bustamante-Filho IC. Structural modeling and mRNA expression of epididymal β-defensins in GnRH immunized boars: a model for secondary hypogonadism in man. *Mol Reprod Dev* 2018;**85**:921–933.

Wehkamp J, Harder J, Weichenthal M, Schwab M, Schaffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P *et al*. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut* 2004;**53**: 1658–1664.

Wehkamp J, Wang G, Kübler I, Nuding S, Gregorieff A, Schnabel A, Kays RJ, Fellermann K, Burk O, Schwab M *et al*. The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *J Immunol* 2007;**179**:3109–3118.

Wiechuła B, Cholewa K, Ekiel A, Romanik M, Dolezych H, Martirosian G. [HBD-1 and hBD-2 are expressed in cervico-vaginal lavage in female genital tract due to microbial infections]. *Ginekol Pol* 2010;**81**:268–271.

Wiechuła BE, Friedek DA, Ekiel AM, Romanik MK, Martirosian G. Human neutrophil peptides in vaginitis/cervicitis of different etiology. *Pol J Microbiol* 2007;**56**:185–189.

Wiens ME, Smith JG. Alpha-defensin HD5 inhibits furin cleavage of human papillomavirus 16 L2 to block infection. *J Virol* 2015;**89**: 2866–2874.

Wiens ME, Smith JG. α-Defensin HD5 inhibits human papillomavirus 16 infection via capsid stabilization and redirection to the lysosome. *mBio* 2017;**8**:e02304–e02316.

Wiesenfeld HC, Heine RP, Krohn MA, Hillier SL, Amortegui AA, Nicolazzo M, Sweet RL. Association between elevated neutrophil defensin levels and endometritis. *J Infect Dis* 2002;**186**:792–797.

Wilde CG, Griffith JE, Marra MN, Snable JL, Scott RW. Purification and characterization of human neutrophil peptide 4, a novel member of the defensin family. *J Biol Chem* 1989;**264**:11200–11203.

Wilmes M, Sahl H-G. Defensin-based anti-infective strategies. *Int J Med Microbiol* 2014;**304**:93–99.

Wilson SS, Wiens ME, Smith JG. Antiviral mechanisms of human defensins. *J Mol Biol* 2013;**425**:4965–4980.

Wira CR, Rodriguez-Garcia M, Patel MV. The role of sex hormones in immune protection of the female reproductive tract. *Nat Rev Immunol* 2015;**15**:217–230.

Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. *Immunol Rev* 2005;**206**:306–335.

Xin A, Cheng L, Diao H, Wu Y, Zhou S, Shi C, Sun Y, Wang P, Duan S, Zheng J *et al*. Lectin binding of human sperm associates with DEFB126 mutation and serves as a potential biomarker for subfertility. *Sci Rep* 2016;**6**:20249.

Xin A, Zhao Y, Yu H, Shi H, Diao H, Zhang Y. Characterization of β-defensin 42 expressed in principal cells at the initial segment of the rat epididymis. *Acta Biochim Biophys Sin (Shanghai)* 2015;**47**: 861–869.

Xin A, Zhao Y, Yu H, Shi H, Liu H, Diao H, Zhang Y. Soluble fusion expression, characterization and localization of human β-defensin 6. *Mol Med Rep* 2014;**9**:149–155.

Xu D, Lu W. Defensins: a double-edged sword in host immunity. *Front Immunol* 2020;**11**:764.

Xu J, Holzman CB, Arvidson CG, Chung H, Goepfert AR. Midpregnancy vaginal fluid defensins, bacterial vaginosis, and risk of preterm delivery. *Obstet Gynecol* 2008;**112**:524–531.

Yamaguchi Y, Nagase T, Makita R, Fukuhara S, Tomita T, Tominaga T, Kurihara H, Ouchi Y. Identification of multiple novel epididymis-specific beta-defensin isoforms in humans and mice. *J Immunol* 2002;**169**:2516–2523.

Yang C, Boone L, Nguyen TX, Rudolph D, Limpakarnjanarat K, Mastro TD, Tappero J, Cole AM, Lal RB. theta-Defensin pseudogenes in HIV-1-exposed, persistently seronegative female sex-workers from Thailand. *Infect Genet Evol* 2005;**5**:11–15.

Yang D, Biragyn A, Kwak LW, Oppenheim JJ. Mammalian defensins in immunity: more than just microbicidal. *Trends Immunol* 2002;**23**: 291–296.

Yang D, Chen Q, Chertov O, Oppenheim JJ. Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. *J Leukoc Biol* 2000;**68**:9–14.

Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schröder JM, Wang JM, Howard OM *et al*. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* 1999;**286**:525–528.

Yang D, Liu Z-H, Tewary P, Chen Q, De La Rosa G, Oppenheim JJ. Defensin participation in innate and adaptive immunity. *Curr Pharm Des* 2007;**13**:3131–3139.

Yano J, Lilly E, Barousse M, Fidel PL. Epithelial cell-derived S100 calcium-binding proteins as key mediators in the hallmark acute neutrophil response during Candida vaginitis. *Infect Immun* 2010; **78**:5126–5137.

Yarbrough VL, Winkle S, Herbst-Kralovetz MM. Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. *Hum Reprod Update* 2015;**21**:353–377.

Yenugu S, Hamil KG, Radhakrishnan Y, French FS, Hall SH. The androgen-regulated epididymal sperm-binding protein, human beta-defensin 118 (DEFB118) (formerly ESC42), is an antimicrobial beta-defensin. *Endocrinology* 2004;**145**:3165–3173.

Yin L, Chung CM, Huo R, Liu H, Zhou C, Xu W, Zhu H, Zhang J, Shi Q, Wong HYC *et al*. A sperm GPI-anchored protein elicits sperm-cumulus cross-talk leading to the acrosome reaction. *Cell Mol Life Sci* 2009;**66**:900–908.

Yu G, Shen FS, Sturch S, Aquino A, Glazer RI, Felsted RL. Regulation of HIV-1 gag protein subcellular targeting by protein kinase C. *J Biol Chem* 1995;**270**:4792–4796.

Yu H, Dong J, Gu Y, Liu H, Xin A, Shi H, Sun F, Zhang Y, Lin D, Diao H. The novel human β-defensin 114 regulates lipopolysaccharide (LPS)-mediated inflammation and protects sperm from motility loss. *J Biol Chem* 2013;**288**:12270–12282.

Yudin AI, Generao SE, Tollner TL, Treece CA, Overstreet JW, Cherr GN. Beta-defensin 126 on the cell surface protects sperm from immunorecognition and binding of anti-sperm antibodies. *Biol Reprod* 2005a;**73**:1243–1252.

Yudin AI, Tollner TL, Treece CA, Kays R, Cherr GN, Overstreet JW, Bevins CL. Beta-defensin 22 is a major component of the mouse sperm glycocalyx. *Reproduction* 2008;**136**:753–765.

Yudin AI, Treece CA, Tollner TL, Overstreet JW, Cherr GN. The carbohydrate structure of DEFB126, the major component of the cynomolgus Macaque sperm plasma membrane glycocalyx. *J Membr Biol* 2005b;**207**:119–129.

Zaballos A, Villares R, Albar JP, Martinez AC, Marquez G. Identification on mouse chromosome 8 of new beta-defensin genes with regionally specific expression in the male reproductive organ. *J Biol Chem* 2004;**279**:12421–12426.

Zaga-Clavellina V, Martha RV-M, Flores-Espinosa P. *In vitro* secretion profile of pro-inflammatory cytokines IL-1β, TNF-α, IL-6, and of human beta-defensins (HBD)-1, HBD-2, and HBD-3 from human chorioamniotic membranes after selective stimulation with Gardnerella vaginalis. *Am J Reprod Immunol* 2012a;**67**:34–43.

Zaga-Clavellina V, Ruiz M, Flores-Espinosa P, Vega-Sanchez R, Flores-Pliego A, Estrada-Gutierrez G, Sosa-Gonzalez I, Morales-Méndez I, Osorio-Caballero M. Tissue-specific human beta-defensins (HBD)-1, HBD-2 and HBD-3 secretion profile from human amniochorionic membranes stimulated with *Candida albicans* in a two-compartment tissue culture system. *Reprod Biol Endocrinol* 2012b;**10**:70.

Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002;**415**:389–395.

Zhang C, Zhou Y, Xie S, Yin Q, Tang C, Ni Z, Fei J, Zhang Y. CRISPR/Cas9-mediated genome editing reveals the synergistic effects of β-defensin family members on sperm maturation in rat epididymis. *FASEB J* 2018;**32**:1354–1363.

Zhao C, Wang I, Lehrer RI. Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. *FEBS Lett* 1996;**396**:319–322.

Zhao S, Zhou HY, Li H, Yi T, Zhao X. The therapeutic impact of HNP-1 in condyloma acuminatum. *Int J Dermatol* 2015;**54**:1205–1210.

Zhao Y, Diao H, Ni Z, Hu S, Yu H, Zhang Y. The epididymis-specific antimicrobial peptide β-defensin 15 is required for sperm motility and male fertility in the rat (*Rattus norvegicus*). *Cell Mol Life Sci* 2011;**68**:697–708.

Zhou CX, Zhang Y-L, Xiao L, Zheng M, Leung KM, Chan MY, Lo PS, Tsang LL, Wong HY, Ho LS *et al*. An epididymis-specific beta-defensin is important for the initiation of sperm maturation. *Nat Cell Biol* 2004a;**6**:458–464.

Zhou N, Fang J, Mukhtar M, Acheampong E, Pomerantz RJ. Inhibition of HIV-1 fusion with small interfering RNAs targeting the chemokine coreceptor CXCR4. *Gene Ther* 2004b;**11**:1703–1712.

Zhou YS, Webb S, Lettice L, Tardif S, Kilanowski F, Tyrrell C, Macpherson H, Semple F, Tennant P, Baker T *et al*. Partial deletion of chromosome 8 β-defensin cluster confers sperm dysfunction and infertility in male mice. *PLoS Genet* 2013;**9**:e1003826.

Zupin L, Polesello V, Martinelli M, Luppi S, Giolo E, Zito G, Romano F, Segat L, Crovella S, Ricci G. Human β-defensin 1 in follicular fluid and semen: impact on fertility. *J Assist Reprod Genet* 2019a;**36**:787–797.

Zupin L, Polesello V, Segat L, Kamada AJ, Kuhn L, Crovella S. polymorphisms and HIV-1 mother-to-child transmission in Zambian population. *J Matern Fetal Neonatal Med* 2019b;**32**:2805–2811.