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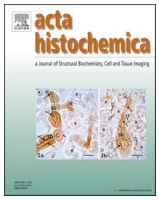

Neuronal control of the vagina in vertebrates: A review

Anna Costagliola [a,*], Giovanna Liguori [a,b], Luc Van Nassauw [c]

[a] Department of Veterinary Medicine and Animal Productions, University of Naples Federico II, Via Delpino, 1, 80137 Naples, Italy
[b] Department of Prevention, ASL FG, Foggia, Italy
[c] Laboratory of Human Anatomy & Embryology, Department ASTARC, Faculty of Medicine & Health Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

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ABSTRACT

Background: At present, there is an increased interest in the vaginal microbiome. It is believed that microbes play equally important roles in the vagina, including the modulation of neuronal pathways, as in the gut. However, in man as well as in animals, the vagina is the least well-studied part of the female reproductive system. The vagina, a fibromuscular tract, having two main functions, i.e., childbirth and sexual intercourse, is mainly innervated by the pudendal nerve and the pelvic splanchnic nerves (the uterovaginal nerve plexus) containing sympathetic, parasympathetic and nociceptive nerve fibers. Innervation density in the vaginal wall undergoes significant remodeling due to hormonally mediated physiological activity. Knowledge about expression and function of neuropeptides and neurotransmitters in the vaginal fibers is incomplete or not established. Most research concerning the neuroregulation of the vagina and the function and expression of neuropeptides and neurotransmitters, is performed in several vertebrate species, including large farm animals, rodents, domestic fowl and lizards.

Methods: This review summarizes, on a bibliographic basis, the current knowledge on vaginal innervation and function of neuropeptides and neurotransmitters expressed in vaginal nerve fibers in several vertebrate species, including humans. The presence and role played by the local microbioma is also explored.

Conclusion: A thorough knowledge of the vaginal innervation is necessary to unravel the putative communication of the vaginal microbiome and vaginal nerve fibers, but also to understand the effects of vaginal pathologies and of administered drugs on the neuroregulation of the vagina.

1. Introduction

Infections and dysfunctions of the genital tract are major worldwide morbidities. The vagina is rich in blood vessels, lymphatics, nerves and a mucosal microbial community. All these components undergo environmental perturbations due to pathogen exposure and physiological fluctuations linked to the reproductive cycle. Gonadal hormones strongly influence the overall structure and function of the vagina (Levy et al., 2020). At present, there is an increased interest in the vaginal microbiome. It is believed that microbes play equally important roles in the vagina, including modulation of neuronal pathways (Levy et al., 2020) as in the gut (Anitha et al., 2012, Grasa et al., 2015, Obata et al., 2020; Vicentini et al., 2021; Yarandi et al., 2020). In the gut, microbiota are involved in a variety of functions. Depletion of gut bacteria alters gastro-intestinal motility, secretion and permeability. Microbiota has been found essential for the maintenance of the integrity of the enteric nervous system, regulating enteric neuronal survival and promoting neurogenesis (Vicentini et al., 2021; Yarandi et al., 2020). A role of gut microbiota in neuropsychiatric disorders or intestinal inflammation, has been suggested by results from both human and animal models. This role seems to be partly mediated by direct or indirect effects of gut neuropeptides on gut microbiota and brain (Aresti Sanz and El Aidy, 2019).

Abbreviations: cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; DBH, dopamine β-hydroxylase; DRG, dorsal root ganglia; GAL, galanin; HRT, hormone replacement therapy; IHP, inferior hypogastric plexus; LSN, lumbosacral spinal nerves; MPG, major pelvic ganglia; NANC, non-adrenergic non-cholinergic; NO, nitric oxide; NOS, nitric oxide synthase; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating polypeptide; PHM, peptide histidine-methionine; PHV, peptide histidine-valine; SP, substance P; TH, tyrosine hydroxylase; VAchT, vesicular acetylcholine transporter; VIP, vasoactive intestinal polypeptide.

* Corresponding author.
E-mail addresses: anna.costagliola@unina.it (A. Costagliola), giovanna.liguori@unina.it (G. Liguori), luc.vannassauw@uantwerpen.be (L.V. Nassauw).

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Understanding the physiological mechanisms responsible for vaginal engorgement, vasocongestion and lubrication responses, is necessary for improving clinical management of female sexual dysfunctions of this segment (Azadzoi and Siroky, 2010). Moreover, a thorough knowledge of the vaginal physiological mechanisms is essential when animals are used as models to study disease mechanisms, dysfunctions, and sexually transmitted diseases of the human genital tract. As for most organ systems, in vivo and in vitro experimental studies of the vagina are mainly performed in animal models. In preclinical studies and in sexual function research, rats and especially mice, due to the availability of genetically modified strains, are predominantly used as experimental animal models (Marson et al., 2013).

Furthermore, farm animals, including cattle, sheep, pigs, and chickens, have been found to be excellent physiological models for studies related to human health or diseases. Farm animals are usually much larger than the commonly used laboratory vertebrate species, making it easier to collect larger volumes of fluids or more tissue biopsies (Ireland et al., 2008). In addition, many studies have revealed that the human physiology is more closely related to the physiology of the large farm animals than to rodents (Hamernik, 2019).

In man, as in the experimental animal models, innervation and neuropeptides are less studied in the vagina, in comparison to the other segments of the female reproductive tract. Most knowledge concerns the autonomic innervation of the vagina, but the knowledge concerning expression and function of neurotransmitters and neuropeptides in the intrinsic vaginal nerve fibers is incomplete, confusing and sometimes not established (Brauer and Smith, 2015; Giuliano et al., 2002). Therefore, a scoping review was conducted in order to collect and to summarize all available data on vaginal innervation and on the presence and actions of neuropeptides and neurotransmitters in nerve fibers of the vaginal wall. These data obtained in several vertebrate species, including humans, will be compared to reveal species similarities and differences. In particular, man, sow, cow, rodents, hen and lizard will be anatomically and physiologically compared and described.

Furthermore, for each species a concise survey of what is known about the vaginal microbiome is included to reveal similarities and differences between species, being useful for future research concerning putative interactions between neuropeptides/neurotransmitters and vaginal microbes.

A schematic representation of the topographic distribution of the

Table 1
Schematic representation of the topographic distribution of the known neurotransmitters and neuropeptides involved in innervation of the vertebrate vagina.

location	*woman*	*sow*	*cow*	*rat*	*mouse*	*hen*	*lizard*
INTRINSIC							
Epithelium	nNOS$^{(F,V)}$ $^{(1)}$VIP$^{(F)}$ $^{(1)}$ PACAP$^{(F,V)}$ (2,3)	TH$^{(F)}$ $^{(4)}$nNOS$^{(F,V)}$ $^{(4)}$VIP$^{(F)}$ $^{(4)}$SP$^{(F)}$ (4)	TH$^{(F)}$ $^{(5)}$SP$^{(F)}$ $^{(5)}$nNOS$^{(F)}$ $^{(4)}$VIP$^{(F)}$ $^{(4)}$SP$^{(F)}$ $^{(4)}$ VIP$^{(V)}$ $^{(5)}$GAL$^{(F,V)}$ (6)	AchE$^{(F)}$ $^{(7,8)}$TH$^{(F,V)}$ $^{(7,8)}$VIP$^{(F)}$ $^{(8)}$ CGRP$^{(F)}$ $^{(8)}$	nNOS$^{(P)}$ $^{(9)}$VIP$^{(P)}$ $^{(9)}$	nNOS$^{(F,V,P)}$ $^{(10)}$VIP$^{(F,V,P)}$ $^{(10,11)}$PACAP$^{(F,V,P)}$ $^{(10,11)}$GAL$^{(F,V,P)}$ (10,11)	GAL$^{(F,V)}$ $^{(12)}$nNOS$^{(F)}$ $^{(12)}$ VIP$^{(F)}$ (12)
Lamina propria	nNOS$^{(F,V)}$ $^{(1)}$PACAP$^{(F,V)}$ (2,3)	TH$^{(F)}$ $^{(4)}$nNOS$^{(F,V)}$ $^{(4)}$SP ($^{F,V)}$ $^{(4)}$GAL$^{(V)}$ (13)	nNOS$^{(F)}$ $^{(4)}$VIP$^{(F)}$ $^{(4)}$SP$^{(F)}$ $^{(4)}$ VIP$^{(V)}$ (5)	nNOS$^{(F,V)}$ (14)	CGRP$^{(F)}$ $^{(9)}$SP$^{(F)}$ $^{(9)}$ nNOS$^{(F)}$ $^{(9)}$VIP$^{(F)}$ (9)	Ach$^{(P)}$ $^{(15)}$nNOS$^{(F,V,P)}$ $^{(10)}$ VIP$^{(F,V,P)}$ $^{(10,11)}$PACAP$^{(F,V,P)}$ $^{(10,11)}$GAL$^{(F,V,P)}$ $^{(10,11)}$SP$^{(F,V,P)}$ (10,11)	GAL$^{(P)}$ $^{(12)}$nNOS$^{(P)}$ $^{(12)}$VIP$^{(P)}$ (12)
Tunica muscularis	Ach ($^{F,V)}$ $^{(16)}$nNOS$^{(F,V)}$ $^{(1)}$VIP$^{(F,V)}$ $^{(1)}$PACAP$^{(F,V)}$ $^{(2,3)}$GAL$^{(F,V)}$ (17)	TH$^{(F)}$ $^{(4)}$nNOS$^{(F,V)}$ $^{(4)}$ VIP$^{(F)}$ $^{(4)}$SP ($^{F,V)}$ $^{(4)}$GAL$^{(V)}$ (13)	TH$^{(F,V)}$ $^{(5)}$nNOS$^{(F,V)}$ $^{(4,5)}$ VIP$^{(F)}$ $^{(4,5)}$SP$^{(F,V)}$ $^{(4,5)}$TH$^{(F)}$ $^{(5)}$SP$^{(F)}$ $^{(5)}$GAL$^{(F)}$ (6)	AChE$^{(F,V)}$ $^{(18)}$nNOS$^{(F,V)}$ $^{(14)}$VIP$^{(F,V)}$ 18,19)TH$^{(F,V)}$ $^{(8,21)}$CGRP$^{(F,V)}$ $^{(8,21)}$VAchT$^{(F,V)}$ $^{(8,21)}$CGRP$^{(F)}$ $^{(8)}$PACAP$^{(F)}$ $^{(8)}$SP$^{(F,V)}$ (8)	CGRP$^{(F)}$ $^{(9)}$SP$^{(F)}$ $^{(9)}$nNOS$^{(F)}$ $^{(9)}$VIP$^{(F)}$ (9)	Ach$^{(P)}$ $^{(15)}$nNOS$^{(F,V,P)}$ $^{(10)}$ VIP ($^{F,V,P)}$ $^{(10,11)}$PACAP$^{(F,V,P)}$ $^{(10,11)}$GAL$^{(F,V,P)}$ $^{(10,11)}$SP$^{(F,V,P)}$ (10,11)	GAL$^{(P)}$ $^{(12)}$nNOS$^{(P)}$ $^{(12)}$VIP$^{(P)}$ (12)
Adventitia	nNOS$^{(F,V)}$ (1)	TH$^{(F)}$ $^{(4)}$NOS$^{(F,P)}$ $^{(4)}$VIP$^{(F)}$ $^{(4)}$SP$^{(F,V)}$ $^{(4)}$GAL$^{(V)}$ (13)	NOS$^{(F)}$ $^{(4,13)}$VIP$^{(F)}$ $^{(4,13)}$SP$^{(F)}$ $^{(4,13)}$GAL$^{(V)}$ (6)	AchE$^{(F,V)}$ $^{(8,18)}$nNOS$^{(F,V,P)}$ $^{(8)}$VIP$^{(F,V)}$ $^{(8,18,19)}$CGRP$^{(F,V)}$ $^{(8)}$PACAP$^{(F,V)}$ (8)	NOS$^{(V)}$ $^{(9)}$VIP$^{(V)}$ $^{(9)}$SP$^{(V)}$ (9)	nNOS$^{(F,V,P)}$ $^{(10)}$VIP$^{(F,V,P)}$ $^{(10,11)}$PACAP$^{(F,V,P)}$ $^{(10,11)}$GAL$^{(F,V,P)}$ (10,11)	
EXTRINSIC							
Sympathetic chain ganglia		NOS$^{(F)}$ $^{(4)}$VIP$^{(P)}$ $^{(4)}$TH$^{(F)}$ $^{(4)}$SP$^{(F)}$ (4)				NA$^{(F)}$ $^{(21)}$	NA$^{(22,23)}$
Preaortic ganglia		NADPH-d$^{(F,P)}$ $^{(4)}$VIP$^{(P)}$ $^{(4)}$SP$^{(F)}$ $^{(4)}$TH$^{(F)}$ (4)					
Inferior mesenteric ganglia		NADPH-d$^{(F,P)}$ $^{(4)}$VIP$^{(P)}$ $^{(4)}$TH$^{(F)}$ (4)		CGRP$^{(F)}$ $^{(27)}$	TH$^{(P)}$ $^{(9)}$NOS$^{(P)}$ $^{(9)}$VIP$^{(F)}$ $^{(9)}$SP$^{(F)}$ (9)		
Major pelvic ganglion		NADPH-d$^{(F,P)}$ $^{(4)}$ VIP$^{(P)}$ $^{(4)}$TH$^{(F)}$ (4)		TH$^{(P)}$ $^{(23)}$ACh$^{(P)}$ $^{(23)}$CGRP$^{(F)}$ $^{(27)}$	TH$^{(P)}$ $^{(9)}$NOS$^{(P)}$ $^{(9)}$VIP$^{(P)}$ $^{(9)}$SP$^{(F)}$ (9)	ACh$^{(28)}$	
Paracervical ganglion	VIP$^{(P)}$ $^{(25)}$PACAP$^{(P)}$ (3,26)	NOS$^{(F,P)}$ $^{(4)}$VIP$^{(P)}$ $^{(4)}$TH$^{(F)}$ $^{(4)}$SP$^{(F)}$ (4)		ACh$^{(F)}$ $^{(27)}$			
Dorsal root ganglia		NOS$^{(F,P)}$ $^{(4)}$SP$^{(F,P)}$ $^{(4)}$VIP (P)$^{(4,13)}$SP (P)$^{(4,13)}$			SP$^{(P)}$ $^{(9)}$VIP$^{(P)}$ $^{(9)}$NOS$^{(P)}$ $^{(9)}$CGRP$^{(P)}$ $^{(9)}$TH$^{(P)}$ (9)		

F: nerve fibres; **V**: perivascular; **P**: perikarya; **Ach:** acetylcholine; **NADPH-d:** nicotinamide adenine dinucleotide phosphate-diaphorase; **nNOS:** neuronal nitric oxide synthase;

References: **1**: Hoyle et al. (1996); **2**: Fahrenkrug et al. (1996); **3**: Graf et al. (1995); **4**: Majewski et al. (1995a); **5**: Lakomy et al. (1995a); **6**: Lakomy et al. (1995b); **7**: Papka et al. (1985); **8**: Giraldi et al. (2002); **9**: Barry et al. (2017); **10**: Costagliola et al. (2004); **11**: Costagliola et al. (1997); **12**: Lamanna et al. (1999); **13**: Majewski et al. (1995b); **14**: Berman et al. (1998); **15**: Gandahi et al. (2013); **16**: Levin (1991); **17**: Bauer et al. (1986); **18**: Papka et al. (1985); **19**: Alm et al. (1980b); **20**: Ting et al. (2004); **21**: Friedman and Sturkie, 1963; **22**: Berger and Burnstock (1979); **23**: Bertrand and Keast (2020); **24**: Wyneken (2007); **25**: Alm et al. (1980a); **26**: Steenstrup et al. (1995); **27** Ghatei et al. (1985); **28**: Giuliano et al. (2002); **29**: King (1975).

known neurotransmitters and neuropeptides involved in innervation of the vertebrate vagina is presented in Table 1.

2. Anatomy of the mammalian vagina

In *Eutheria* (or *Placentalia*), the upper or main part of the vagina is formed by an unpaired, median tube, originating from the most caudal part of the paramesonephric (Mullerian) ducts. It is located in the pelvic cavity, and joins the vaginal bulb of the urogenital sinus. Residual parts of the mesonephric (Wolffian) ducts fuse with the dorsolateral wall of the vagina. The vagina or female copulatory organ receives the male copulatory organ during copulation, and is the birth canal for the offspring during parturition (Kurita, 2010; McCracken et al., 2021). In all species, the vagina consists of three layers: a mucosa, an intermediate muscular layer and an outer serosal or adventitial layer (Barone, 2008; Eurell and Frappier, 2006). The *tunica muscularis* comprises an inner circular and outer longitudinal muscle layer. The serosal layer faces the peritoneal and pelvic cavities (Eurell and Frappier, 2006). In the peritoneal cavity, the cranial part of the vagina is covered by the *tunica serosa*, while in the pelvic cavity, the outer layer only contains loose connective tissue (*tunica adventitia*) (Eurell and Frappier, 2006). Both *tunica serosa* and *adventitia* contain large vascular and lymphatic plexuses, numerous nerve bundles and ganglia (Bacha and Bacha, 2000; Eurell and Frappier, 2006; Krstic, 2013).

2.1. The human vagina

2.1.1. Anatomy

In women, the vagina is a fibromuscular segment that extends from the cervix to the vulva. It is H-shaped in its central portion (Sokol and Shveiky, 2008). The posterior vaginal wall is almost 10 cm long. The anterior vaginal wall, incorporating the cervix, has almost the same length (Sokol and Shveiky, 2008). Its wall presents a series of transverse epithelial ridges (different in rodents) called *rugae*, produced by folding of the wall of the outer third of the vagina. *Rugae* allow extension and stretching during sexual intercourse and parturition (Krause, 2005; Squier et al., 2008).

The vaginal *lamina epithelialis* is a non-keratinized stratified squamous epithelium that responds to ovarian steroid hormone cycling (Eurell and Frappier, 2006). The mucosal surface is protected by an acid environment resulting from bacterial growth on glycogen present in the vaginal mucus (Wilson et al., 2010). The *lamina propria* is a thin layer of elastic fibers with a dense vascular network and contains no glands or smooth muscle cells (Eurell and Frappier, 2006). The vaginal lubrication is provided by transudate from the blood vessels and secretions of the Bartolin's glands near the vaginal opening and the cervix. The submucosal layer contains several large veins. The muscle layer comprises an inner smooth muscle layer, circumferentially oriented and an outer smooth muscle layer, longitudinally oriented. The external fibrous capsula is rich in elastic fibers and contains vessels, lymphatics and nerves (Krstic, 2013; Pauls et al., 2006).

The autonomic innervation of the vagina is provided by the pelvic splanchnic nerves, and the hypogastric and paravertebral sympathetic chains. Preganglionic parasympathetic fibers originate in the sacral parasympathetic nucleus. Inhibitory sympathetic fibers originate in the dorsal gray commissure and the intermediolateral cell column at the thoracolumbar level. The activity of these spinal nuclei is controlled by descending projections from the brain. The postsymphathetic and parasympathetic ganglia reside in the inferior hypogastric plexus (IHP: synonyms are bilateral major pelvic ganglia, paracervical ganglia, Frankenhäuser's ganglia (Bertrand and Keast, 2020). Sensory afferents pass through the pudendal, hypogastric, and IHP from the vagina (Bertrand and Keast, 2020; Giuliano et al., 2002). The nerves are associated with vascular and non-vascular smooth muscle and with epithelia, thus mainly regulating blood flow, lubrication, nociception and the vaginal smooth muscle tone, contributing to maintain tissue integrity (Jobling, 2011). Some intraepithelial nerve fibers are present at the vaginal orifice. However, the vaginal epithelium proximal to the distal opening, appears largely devoid of nerve fibers (Hilliges et al., 1995).

The blood supply is provided by the vaginal artery that anastomoses with vaginal branches from the uterine, middle rectal and internal pudendal arteries, all ramifications of the internal iliac artery just as the vaginal artery. Arterial branches pass through the *tunica muscularis* and form a mucosal plexus within the *tunica propria*. The veins are numerous and large. Venous blood passes along the small subepithelial veins into the veins of the muscular layer, which drain into the veins of the external layer. Veins join the venous plexuses on the pelvic floor, which drain into the internal iliac vein. The lymphatics of the vagina drain into the external and internal iliac lymph nodes and into the superficial inguinal lymph nodes (Łaniewski and Herbst-Kralovetz, 2018).

2.1.2. Neurotransmitters and neuropeptides

Despite a rich cholinergic innervation of the vaginal arteries, acetylcholine plays a minor role in the regulation of vaginal blood flow, in contrast it stimulates the vaginal smooth muscle contraction (Levin, 1991). Nitric oxide synthase (NOS)-expressing or nitrergic nerve fibers are found throughout the genital tract. In the vagina, nitrergic nerves are found in the vicinity of vascular and non-vascular smooth muscle, and in the subepithelial plexus beneath the epithelium. Their density increases distally, and it is suggested that nitric oxide (NO) plays a role in vaginal contractile activity, blood flow, and epithelial permeability and transport. Nitrergic nerves innervate the arteries and veins in the adventitia more extensively than those in the *lamina propria* (Hoyle et al., 1996). NO activity has been found highest in the vagina of non-pregnant women (Hoyle et al., 1996). The vaginal NOS activity in human, is considerably higher than in the myometrium and is downregulated by estrogen (Batra et al., 2003). NO probably also has a sensory role, because neuronal NOS has been found in a subpopulation of sensory neurons in dorsal root ganglia (DRG) of humans (Terenghi et al., 1993). Therefore, NO may be involved in the vasocongestion of the vagina and in the relaxation of the inner vaginal wall (Hoyle et al., 1996).

Vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) belong both to the glucagon hormone superfamily. The highest density of VIP-expressing or VIPergic nerve fibers in the female reproductive tract, is located in the cervix and vagina, in the vicinity of vascular and non-vascular smooth muscles (Blank et al., 1986; Graf et al., 1995; Helm et al., 1981; Hoyle et al., 1996; Lynch et al., 1980; Palle et al., 1989). It is observed that VIPergic nerve fibers form a subepithelial plexus, without penetrating the epithelium (Hoyle et al., 1996). It is assumed that the vaginal effect of VIP is increasing vaginal blood flow and eliciting vaginal lubrication (Levin, 1991; Ottesen et al., 1983, 1987). Postmenopausal women receiving estradiol replacement and VIP, exhibit an increased vaginal blood flow in comparison to premenopausal women (Goldstein et al., 2005). In women, VIP-expressing nerve cell bodies are detected in the paracervical ganglion (Alm et al., 1980a; b).

PACAP is an ancient and well-conserved peptide that exists in a 38-amino acid form and a truncated 27-amino acid form that is identical to the N-terminal portion of PACAP-38 (Sherwood et al., 2000). PACAP-38-expressing nerves are more abundantly present in the human female genital tract than PACAP-27-containing nerves (Fahrenkrug et al., 1996). PACAPergic nerve fibers were detected throughout the vaginal wall, more abundantly in the submucosa where a dense nerve network runs below the mucosal epithelium, in the *lamina propria* around veins, and in the wall of blood vessels of the smooth muscle layer. This localization of PACAPergic nerve fibers suggests an involvement of PACAP in blood flow and lubrication and less in the innervation of the vaginal smooth muscle (Fahrenkrug et al., 1996, 2003; Graf et al., 1995). These PACAPergic nerve fibers arise from neuronal cell bodies located in the paracervical ganglion (Graf et al., 1995; Steenstrup et al., 1995), and they co-store VIP (Graf et al., 1995).

PACAP is also expressed in about half of VIPergic nerve fibers present in the mucosal epithelium (Graf et al., 1995). It is reported that PACAP inhibits the spontaneous smooth muscle activity of human myometrial strips and myometrial arteries precontracted with norepinephrine with an activity similar to that of VIP (Fahrenkrug et al., 1996, 2003; Steenstrup et al., 1995).

In humans, galanin (GAL) is a 30-amino acid peptide (Tatemoto et al., 1983), that is not amidated on the C-terminus. The N-terminal part of GAL is highly conserved throughout evolution. GAL acts primarily as a modulator of neuronal and non-neuronal functions (Lang et al., 2015). GAL-expressing nerve fibers are found within the smooth muscle and in close relationship to blood vessels in the vagina and cervix of women (Bauer et al., 1986). Functional studies have shown that GAL exerts contractile effects and only modulates certain types of smooth muscle preparations but not smooth muscle of large veins and arteries (Ekblad et al., 1985). More recently, an emerging role for GAL has observed in innate immunity, inflammation, and cancer (Lang et al., 2015).

The expression of other neuropeptides (i.e., calcitonin gene-related peptide (CGRP), a faint quantity of substance P (SP), neuropeptide Y (NPY), peptide histidine-methionine (PHM), peptide histidine-valine (PHV), and helospectin is also demonstrated in vaginal nerve fibers, although data concerning co-expression are lacking (Bauer et al., 1986; Blank et al., 1986; Jorgensen et al., 1989; Graf et al., 1995; Hoyle et al., 1996; Palle et al., 1990, 1992; Steenstrup et al., 1995). NPY-expressing nerve fibers are located in the vicinity of vascular and non-vascular smooth muscle (Blank et al., 1986; Jorgensen et al., 1989). Intravaginal injections of PHM in women, increase vaginal blood flow (Palle et al., 1990).

2.1.3. Effects of estrogen

Ovarian steroids are important in maintaining the homeostasis of the vaginal tissue. Imbalances in the hormonal milieu contributes to vaginal pathophysiology. Menopausal phase in women causes several structural changes such as shortening and narrowing of the vagina, and loss of vaginal folds with advancing age. The epithelial layer flattens, keratinizes, and decreases in diameter (Forsberg, 1995). Estrogen is important for the maintenance and function of the vaginal epithelium, stromal cells, smooth muscles and nerve trophism. Estrogen has vasodilatory effects and increases vaginal, clitoral and urethral blood flow via NOS and VIP pathways, leading to genital congestion and vaginal lubrication. Estrogen also modulates sensory thresholds (Al-Azzawi et al., 2010; Goldstein et al., 2005). Estrogen deprivation, occurring during the menopausal stage, may led to decreased pelvic blood flow, followed by reduced vaginal lubrication, thinning of the vaginal wall, decreased vaginal submucosal vasculature and elevated vaginal pH. In contrast, estrogen replacement in postmenopausal women increases pelvic blood flow, re-establishing vaginal integrity and lubrication (Goldstein et al., 2005).

Altered estrogen levels also affect the vaginal innervation. In postmenopausal women, hormone replacement therapy (HRT) results in a decreased innervation density in the whole vaginal wall. Affected populations include tyrosine hydroxylase (TH)-expressing sympathetic fibers, VIPergic presumptive parasympathetic axons, and non-adrenergic non-cholinergic (NANC) fibers that presumably represent both peptidergic (i.e., CGRP-expressing) and non-peptidergic afferent fibers. Topical HRT has been found more effective than systemic HRT in reducing vaginal innervation (Griebling et al., 2012), which is consistent with the idea that this effect is due to direct actions of estrogen on the target tissue rather than being secondary to the estrogen effects.

2.1.4. Vaginal microbiome

The woman's vaginal microbiota may influence conception, pregnancy, mode and timing of delivery, and the risk of acquiring sexually transmitted infections (Amabebe and Anumba, 2018). A healthy vaginal microbioma contains more *Lactobacilli*, oxygen-tolerant anaerobes that contribute to the formation of a physical barrier against pathogen adhesion, that stimulate host defences, and that produce lactic acid having antimicrobial activity against a range of vaginal pathogens (Tachedjian et al., 2017). The composition of the vaginal microbiota is dynamic and changes on hormonal fluctuations throughout the reproductive life. In puberty and pregnancy, estrogen control of the vaginal microbiota promotes and preserves the homeostasis of the vaginal microenvironment (Smith and Ravel, 2017). Estrogen stimulates maturation and proliferation of the vaginal epithelia and their accumulation of glycogen. The higher content of glycogen favorites the growth of *Lactobacilli* which produce lactic acid. Lactic acid decreases the vaginal pH that protects the vaginal mucosa against infections (Łaniewski and Herbst-Kralovetz, 2018; O'Hanlon et al., 2013; Tachedjian et al., 2017), *Lactobacilli* may also generate NO by by either the NOS-mediated synthetic process or the nitrate/nitrite-NO pathway. NO has an important role in the vaginal innate immune response to the condition of microbial dysbiosis known as bacterial vaginosis, and so, in maintaining gynecological homeostasis (Tiso et al., 2015; Yeruva and Lee, 2019). Modification of the vaginal microbiome (vaginal dysbiosis) causes preterm birth, pelvic inflammatory diseases, infertility, or even gynecological cancers (Rowe et al., 2020).

2.2. The porcine vagina

Among animal farms, the pig (or the Göttingen minipig) is a suitable animal model for human diseases because of the physiological and anatomical similarities with humans (Lorenzen et al., 2015). In addition, the genital tract of woman and sow share more genes and proteins than with the female genital tract of mice (McAnulty et al., 2011). The reproductive and the hormonal cycles under control of the hypothalamic-pituitary-ovarian axis, are closely related and only slighty differ in cycle duration (Senger, 2005; Silverthorn, 2007).

2.2.1. Anatomy

In the pig, the vagina length is approximately 13 cm, while in minipig, it is 8 cm (Lorenzen et al., 2015). The histology of the vaginal segment is very similar to that of women. It consists of three layers: the mucosal, the muscular and the outer serosal layer (Eurell and Frappier, 2006). The vaginal *lamina epithelialis* is a non-keratinized stratified squamous epithelium that forms longitudinal folds called *rugae* as in humans (Eurell and Frappier, 2006; Squier et al., 2008). The porcine vaginal epithelium undergoes cyclic alterations reaching a maximum thickness in the late proestrus (Eurell and Frappier, 2006). The vaginal mucosa is moisturized with secretions from the cervix. As in humans, a dens network of blood vessels is located in the *lamina propria* while no glands or smooth muscle cells are present in this layer (Eurell and Frappier, 2006). The submucosal layer contains several large veins, and the *tunica muscularis* comprising an inner circular and an outer longitudinal muscle layer, is similar as in humans. However, in pig, a thin layer of longitudinally oriented smooth muscle fibers, may be observed in the circular layer (Bal and Getty, 1972; Barone, 2008; Eurell and Frappier, 2006). Both *tunica serosa* and *adventitia* contain large blood vessels, extensive venous and lymphatic plexuses, numerous nerve bundles and ganglia with a topographic distribution similar to that observed in humans (Bacha and Bacha, 2000; Eurell and Frappier, 2006).

2.2.2. Neurotransmitters and neuropeptides

Adrenergic nerves are numerous in the porcine vagina (Majewski et al., 1995a). As in the human vagina, the vaginal wall of pig contains numerous VIPergic and nitrergic nerves, more than elsewhere in the genital tract. VIPergic nerve fibers innervate both vascular and non-vascular smooth muscle, and epithelial cells. Nitrergic axons reach the periphery of the vaginal wall as nerve bundles arising from nerve trunks located under the serous membrane (proximal part) and in the adventitial layer (distal part) of the vaginal tunica propria. In both the outer (longitudinal) and the inner (circular) layer of the tunica

muscularis, nitrergic axons are moderate in number, following the length axis of the smooth muscle bundles. Vaginal arterial vessels are supplied by numerous fine varicose nitrergic nerve fibers. The vaginal mucosa contains many perivascular and non-vascular nitrergic nerve fibers. Delicate varicose nitrergic nerves are also found beneath the vaginal epithelium (Majewski et al., 1995a). In pig, adrenergic nerves do not express NOS, while, as in human, co-expression of NOS and VIP is observed in vaginal nerves, in the paracervical ganglia and in a few sensory neurons of DRG. NOS also frequently co-localizes with SP. However, subsets of nerve fibers, either only containing NOS or SP, are present throughout the vaginal wall. (Majewski et al., 1995a; b). A perivascular nerve plexus, containing VIP and SP, is formed around the vaginal artery (Majewski et al., 1995b). In pig as in other species but not in human, GAL is 29-amino acide peptide being amidated on the C-terminus (Tatemoto et al., 1983). Few GAL-expressing perivascular nerves are found around the vaginal arteries. They are not adrenergic but a few co-express SP (Majewski et al., 1995b). Administration of a mixture of estradiol and progesterone to ovariectomized pigs cause an increase in vaginal norepinephrine content (Kaleczyc, 1994).

2.2.3. Vaginal microbiome

Lactobacilli are more abundant present in the vagina of prepubescent minipigs in comparison to sexually mature non-pregnant animals. In contrast to women, the vaginal microbiome is stable before and after sexual maturity and after estrous cycle. Nevertheless, lactobacilli do not dominate the vaginal microbiota as in women. These differences between women and pigs have to be considered when minipigs are used as experimental model in studies of the female genital tract (Lorenzen et al., 2015).

2.3. The bovine vagina

Based on similarities in ovarian follicular dynamics and endocrine control in cattle and women, the bovine model is used for studying reproductive events, particularly concerning reproductive aging (Mahli et al., 2005).

2.3.1. Anatomy

The length of the bovine vagina is approximately 30 cm. As in all female mammals, the mucosa is yellow-pink during the anestrus periods, and is red and congested during the estrus period. It is covered with mucus mainly produced by cervical mucosa as in human and sow (Elstein, 1982). In cow, *rugae* formed by the epithelium, are found close to the uterine neck. The mucosa is a stratified epithelium containing some goblet cells and is infiltrated by a few lymphocytes and granulocytes. The epithelium is low during the diestrus phase and becomes tall during the estrus phase and desquamates (Barone, 2008). The lymphatic supply is organized into three networks (located in the *lamina propria*, the *tunica muscularis*, and the perivaginal region) draining into the internal iliac lymph nodes (Barone, 2008).

2.3.2. Neurotransmitters and neuropeptides

The sympathetic nerve supply comprises adrenergic nerves, which are numerous in the bovine vagina. TH- and dopamine β-hydroxylase (DBH)-expressing nerve branches of the hypogastric nerve, innervate the circular muscle layer. Some of these branches traverse the muscular layer. Peri- and paravascular adrenergic nerves are also present. Fine nerve terminals are located beneath the epithelium (Lakomy et al., 1995a; b). During pregnancy, the number of vaginal adrenergic nerves do not reduce, and they are involved in the inhibition of the local musculature (Stjernquist and Owman, 1990). These adrenergic nerves and sacral parasympathetic nerves arise from the pelvic plexus. The parasympathetic nerves are connected to small paravisceral ganglia. From the vaginal plexus arise motoric nerves to the muscular layer of the vaginal wall and to the vaginal mucosa surrounding epithelial cells (Barone, 2008).

VIPergic nerves are abundant in the vagina as observed in humans (Alm et al., 1980a; b). They comprise peri- and paravascular nerves as in rat and guinea-pig, nerves to the muscular coat, and nerve fibers to the lamina propria and to the epithelium. The vagina contains a rich supply of GAL-expressing nerves that innervate blood vessels, the muscle coat, and the mucosa where nerve terminals extend between epithelial cells. The majority of GAL-containing nerves are adrenergic. Some GAL-expressing nerves contain also VIP or SP (Lakomy et al., 1995b). A rich supply of SP-containing nerves is also present. SP-expressing nerves run around blood vessels, along smooth muscle bundles and below the epithelium (Lakomy et al., 1995a; b). As in humans and rodents, SP acts as a vasodilator increasing blood flow in the reproductive organs (Gram and Ottesen, 1982). They may also act as primary sensory neurons (Huang et al., 1984). SP- and VIP-containing nerve fibers also occur around the adventitia of the vaginal artery (Majewski et al., 1995a; b). SP may also play a role in neurohormonal reflexes during copulation and lactation (Papka et al., 1985; Traurig et al., 1984). Nitrergic nerves do not co-localize with catecholamines in the bovine vagina. In cows, like in pig, NOS frequently co-localizes with SP and VIP (Majewski et al., 1995a).

2.3.3. Vaginal microbiome

In cattle, the vaginal microbiota is influenced by the proximity of the gastro-intestinal tract. It shows a great bacterial diversity, in contrast to the vaginal microbiota in women, and low diversity for the archaeal and fungal components. Studies at phylum and genus levels show that the most abundant bacterial phyla are *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. As in women, a great microbial variety in the vaginal community among animals is observed. It was suggested that the vaginal microbiome is poorly influenced by hormones during puberty and pregnancy (Laguardia-Nascimento et al., 2015), which was contradicted by more recent research indicating that the estrus cyclus influences the vaginal microbial community in heifers (Deng et al., 2019; Messman et al., 2020; Wang et al., 2019). *Lactobacillus spp.*, although prevalent, is not the abundant genus in cow, in contrast to the human vaginal microbiota. The near-neutral pH observed in cows is consistent with the observed low abundance of *Lactobacillus spp.* (Swartz et al., 2014).

2.4. The rodent vagina

Rats and mice are usually used as animal models for sexual function research (Marson et al., 2013). They are polyestrous and spontaneous, not induced, ovulators.

2.4.1. Anatomy

Unlike the human vagina, the rodent vagina is short and muscular (McCracken et al., 2021). The mucosa is lined by a keratinized stratified squamous epithelium that modifies its morphology during the estrus cycle. The vaginal mucosa is folded with no glands. The *lamina propria* mainly contains connective tissue, and the smooth muscle layer consists of a circular and longitudinal layer that change in density along the length of the vagina, suggesting that proximal, middle and distal vaginal regions have distinct functions (Skoczylas et al., 2013). The external vaginal layer is the *tunica adventitia* that is continuous with the rectal and urethral adventitial layer. Some differences are reported concerning the density of the vaginal innervation in rats in comparison to the innervation density in human. In rat, the innervation density increases toward the distal part of the vagina (Giraldi et al., 2002), while in human the density remains consistent throughout the vaginal wall (Pauls et al., 2006).

2.4.2. Neurotransmitters and neuropeptides in rat

In rat, density of the vaginal innervation appears homogenously distributed along the length of the vagina. However, a higher density of cholinergic nerves in the proximal part of the vagina was observed (Skoczylas et al., 2013). The circumferential sphincteric muscle of the

distal portion of the vagina, supporting the vaginal opening, is thicker and is mainly stimulated by adrenergic agonists (Skoczylas et al., 2013). The vaginal innervation supply is composed of postganglionic sympathetic and parasympathetic neurons, that arise from the bilateral major pelvic ganglia (MPG), also named IHP (human), pelvic ganglia, paracervical ganglia or Frankenhäuser's ganglion (Bertrand and Keast, 2020), and of sensory nerves from lumbosacral DRG that travel via the MPG. The sympathetic nerves originate from the L2-L4 lumbar splanchnic nerves and pass via the hypogastric nerves, while the parasympathetic nerves run through the pelvic nerves (Giuliano et al., 2002). MPG lay between the uterine body and the cranial part of the vagina. These ganglia receive the pelvic nerves at their dorsal edge, the cavernous nerve at their most caudal corner, the hypogastric nerve at their cranial edge, and the accessory nerves at their ventral edge (Bertrand and Keast, 2020). Nitrergic axons, and NPY-, VIP- and PACAP-containing nerve terminals to the vaginal wall also occur (Huang et al., 1984; Giraldi et al., 2002; Polak and Bloom, 1984; Ting et al., 2004). The rodent vagina contains less VIPergic nerves than the human vagina, and they do not contact the epithelium (Alm et al., 1980b; Papka et al., 1985), as observed in human (Hilliges et al., 1995; Ting et al., 2004). In the central region of the rat vagina, nerves containing TH, CGRP and vesicular acetylcholine transporter (VAchT) are present in similar proportions (Ting et al., 2004). These fibers innervate the prominent vaginal smooth muscle layer and the blood vessels. CGRP-expressing fibers are derived from pelvic and hypogastic nerves (Ghatei et al., 1985). Nitrergic nerves innervate the arteries and veins in the adventitial layer more extensively than in the lamina propria. The NO-cyclic guanosine monophosphate (cGMP) pathway is involved in the nerve-stimulated vaginal blood flow in rat (Berman et al., 1998).

Functional studies in rat provided evidence that sympathetic nerves are excitatory to vaginal non-vascular smooth muscle (Gunn and Franklin, 1922), while nitrergic nerves appear to mediate muscle relaxation and vasodilation (Munarriz et al., 2003; Giraldi et al., 2002; Giuliano et al., 2002). The vaginal NOS activity in rat, is considerably higher than in the myometrium (Batra et al., 2003). CGRP-expressing sensory nerves also inhibit vaginal smooth muscle (Giraldi et al., 2002), and they presumably mediate sensations of discomfort associated with vaginal irritation or distension (Berkley et al., 1995). While some intraepithelial nerve fibers are present at the vaginal orifice, the vaginal epithelium appears largely devoid of any innervation proximal to the introitus or vaginal opening in both rat and human (Hilliges et al., 1995; Ting et al., 2004).

2.4.3. Effects of estrogen

Estrogen regulates the vascular components of the genital tissues by regulating the activity of VIP and neuronal and endothelial NOS in the vagina. In rat, the total amount of NOS in the vagina is downregulated by estrogen (Al-Hijji et al., 2000; Batra and Al-Hijji, 1998; Berman et al., 1998), but upregulated by progesterone. However, estrogen upregulates endothelial NOS in the vagina (Goldstein et al., 2005). Unlike in the uterus, the vaginal innervation is not affected by short-term changes in serum estrogen levels. Hence, cyclical variations in serum estrogen levels do not result in detectable changes in any population of vaginal axons in the adult nulliparous rat. It is shown that a single acute injection of 17β-estradiol, sufficient to deplete uterine innervation, does not alter vaginal innervation in adult ovariectomized rats. Thus, vaginal innervation is apparently more resistant to short-term effects of elevated estrogen (Liao and Smith, 2011).

However, prolonged estrogen elevations result in depletion of sympathetic nerves, as also observed in the uterus. Implantation in ovariectomized rats of estrogen-releasing pellets, in order to produce estrogen elevations as detected at term pregnancy, causes a significant reduction in the total number of axons, especially the sympathetic DBH-expressing axons (Zoubina et al., 2001). In addition, chronic exposure to estrogen is also accompanied by depletion of cholinergic axons and CGRP-containing nociceptive afferents in the vagina, but not in the uterus (Ting et al., 2004). A similar depletion has been observed in the vaginal innervation of rats at term pregnancy, at 21 days, when serum estrogen has been elevated for several days, in comparison to pregnant rats at 10d post-coitus when estrogen levels are low (Liao and Smith, 2011).

In rat, ovariectomy causes in the vagina, a significant decrease in the epithelial height, reduces the volume of the muscular layer, and stimulates the deposition of connective tissue between smooth muscle cells. Conversely, estradiol treatment in ovariectomized animals increases the thickness of the muscle layer and reduces connective tissue production (Pessina et al., 2006). Contrasting results on the effects of ovariectomy followed by estradiol treatment in rat, have been observed on the density or distribution of vaginal innervation. Ovariectomy reduces smooth muscle relaxation to electric field stimulation and to exogenous VIP in organ bath, but also estrogen treatment of ovariectomized animal reduces the relaxation response. Estrogen treatment increases the noradrenaline content of the adrenergic nerves but reduces the innervation density in the vagina of ovariectomized rats (Goldstein et al., 2005).

2.4.4. Neurotransmitters and neuropeptides in mouse

In mouse, nerve fibers are distributed along the entire length of the murine vagina, as in human and rat (Vrbanac et al., 2018). Nerve fibers in the epithelium are more numerous distally in the vagina, and never reach the vaginal lumen. They contain CGRP, SP, or VIP and rarely neuronal NOS, but not NPY. Epithelial innervation is diminished in older mice, particularly those releasing VIP (Barry et al., 2017). This may mediate vaginal lubrication as observed in human (Ottesen et al., 1987). In contrast, the *lamina propria* contains a dense network of fibers expressing CGRP, VIP, SP or neuronal NOS. They are more abundant in the proximal part compared to the distal part, as in rat. Nerve fibers containing NPY are rare in the *lamina propria* and predominantly present around small arterioles located close to the circular muscle layer, as well as the adrenergic nerve fibers. The smooth muscle layer contains a few large nerve bundles and a few single nerve fibers. They express VIP, CGRP, SP, or neuronal NOS, whereas NPY-expressing nerve fibers are rare. The adventitial layer contains bundles of nerve fibers and single nerve fibers adjacent to arterioles but not venules. Many perivascular nerves express VIP and neuronal NOS. Other fibers adjacent to blood vessels express CGRP/SP or CGRP/VIP. NPY-containing nerves are predominantly present in the *tunica adventitia*. Some of these fibers are adjacent to blood vessels. Ganglia are detected in the proximal part of the vagina. The ganglionic perikarya express VIP and neuronal NOS. A few somas express NPY. In mouse, VIP/NOS-containing neurons are mainly localized in pelvic ganglia rather than in DRG or inferior mesenteric ganglion (Barry et al., 2017).

2.4.5. Vaginal microbiome

The vaginal microbiota of rat and mouse (Vrbanac et al., 2018) are similar to that of dog (Lyman et al., 2019), and pig (Lorenzen et al., 2015), but different from the baboon vaginal microbiota (Uchihashi et al., 2015). The dominating phyla in the rodent and porcine vaginal microbiome are *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (Levy et al., 2020). Moreover, rat, mice and monkeys have a vaginal flora that is dominated by *Streptococcus spp.* and *Corynebacterium spp.*. A recent study in rat reported that the rat vaginal microbiome has a dynamic microbiome which may change based on the estrous cycle, being unique compared to other animals. No domination of *Lactobacillus spp.* as in the human vaginal microbiome, was observed. This study was the first that examined the effect of neuronal stimulation on the vaginal microbiome. The electrical stimulation of the genital branch of the pudendal nerve did not modify the vaginal microbiota across the estrous cycle but rather effected its stability in healthy rat. The authors suggested that neuronal stimulation may have a potential effect on vaginal microbiome diversity (Levy et al., 2020).

2.5. The avian vagina

Almost all data concerning reproductive organs in birds, are obtained from domestic species, like the domestic fowl (*Gallus domesticus*) and the Japanese quail (*Coturnix japonica*). Birds are oviparous, and only the left ovary and oviduct are developed. The oviduct is divided into five major sections: the infundibulum, magnum, isthmus, uterus (or shell gland), and vagina. The oviduct may be compared to an assembly line. It begins with the deposition of the albumen around the fertilized or unfertilized ovum. Next, the shell membrane accrues, and lastly the shell. Prior to oviposition, the bloom or cuticle is formed on the egg forming a barrier for microorganisms, reducing evaporative loss, and being water repellent. At the utero-vaginal junction, a narrow band at the cranial end of the vagina, the sperm storage tubules are located (Bakst, 1998). The vagina is also involved in the selection, transport and storage of sperm (Proudman, 2000).

2.5.1. Anatomy

The vagina of the domestic fowl is about 12 cm long. It contains several layers: an epithelium, a lamina propria, a poorly defined submucosa, a spirally arranged circular and longitudinal muscle layer, and a serosa. Neuronal cell bodies occurring singly or in groups of two or three cells, are located at peritoneal side of the muscular layer and in the submucosa (Biswal, 1954). The vaginal epithelium is pseudostratified and densely populated with ciliated cells. Specialized tubular infoldings of the epithelium occur in the cranial part of vagina (sperm-host glands at the utero-vaginal connection) that are involved in the survival of spermatozoa in the oviduct (Lake, 1975). The mucosa is arranged in a series of high and narrow primary folds. These folds are divided into smaller secondary and tertiary folds which involve the epithelium only (Bakst, 1998).

The arterial supply of the vagina is provided by the vaginal artery. It is a short arterial branch of the pudendal artery or small branch passing to the shell gland. (Freedman and Sturkie, 1963a; Hodges, 1965). The main artery forms a plexus between the two muscle layers and send branches to a secondary arterial plexus in the lamina propria. From here the arterioles pass through the centre of the mucosal folds forming a capillary network beneath the epithelium (Gilbert, 1979).

The nerves supplying the oviduct, including the vagina, orginate from the left side of the avian body. The vaginal innervation includes sympathetic and parasympathetic nerves. The sympathetic vaginal innervation is supplied by branches of the hypogastric nerve, a continuation of the aortic plexus (Freedman and Sturkie, 1963b). The parasympathetic pelvic nerves that constitute the left pelvic plexus arise from pelvic visceral rami of lumbosacral spinal nerves (LSN 8–11) (King, 1975). From the largest ganglion in the pelvic plexus, i.e., the utero-vaginal ganglion, rami arise which innervate the vagina (Gilbert and Lake, 1963).

2.5.2. Neurotransmitters and neuropeptides

An intrinsic innervation network, being more extensive in the shell gland and vagina, extends along the whole oviduct. It consists of plexuses located beneath the serosa, in the *tunica muscularis*, and in the mucosa. These plexuses are ganglionated in the domestic fowl (Biswal, 1954; Costagliola et al., 1997, 2004; Gilbert and Lake, 1963). A few cholinergic neurons have been found in the intermuscular region and the *lamina propria* of the vagina (Gandahi et al., 2013). Nitrergic neurons are observed in all layers from the adventitia through the intermuscular space to the mucosa, being more numerous in the inner smooth muscle layer facing the *lamina propria*. Para- and perivascular nitrergic nerve fibers are also present. Transmural nerve stimulation of the segment evokes a relaxation which is blocked by L-nitroarginine confirming nitrergic neurotransmission (Costagliola et al., 2004). The vaginal intrinsic neurons express also the neuropeptides VIP, PACAP and GAL with a similar distribution pattern and partial co-expression (Costagliola et al., 1997, 2004; Sakamoto et al., 2000). GAL contracts vaginal muscle strips (Costagliola et al., 2004). VIP and PACAP directly induce relaxation of the vaginal smooth muscle, acting via a common receptor, the VPAC2 receptor (Costagliola et al., 2004). TH-expressing nerve fibers are observed in the *tunica muscularis* (Costagliola et al., 2004).

2.5.3. Vaginal microbiome

Unlike humans, where the vaginal and upper reproductive tract microbiota are significantly different, the mature hen oviduct microbiota does not show significant differences along the tract. *Lactobacillus* species are detected in the mature hen oviduct. Their relative abundance is lower than in the mature human vaginal microbiota but is higher than in other mammalian species (Lee et al., 2019). Differently from humans, the *Lactobacillus* species in the chicken oviduct are not transferred to the offspring suggesting a different role in the reproductive tract between both species (Dominguez-Bello et al., 2010).

2.6. The lizard vagina

The reptilian oviduct contains all structures of the female reproductive apparatus developed from the embryonic Mullerian duct (Wake, 1985). In most reptiles, the oviduct is a paired structure, laying dorsally against the abdominal wall (Fox, 1997). Reptiles include four living orders (*Crocodilia*, *Sphenodontia*, *Squamata*, and *Testudines*), and their oviduct has multiple functions reflecting the variety of parity modes of the diverse reptilian groups that are oviparous or viviparous. In viviparous reproduction, the embryo is retained within the uterus until development is complete and birth occurs (parturition) at the end of the gestation, as in mammals. In contrast, oviparity is characterized by the laying of eggs containing embryos that develop outside the female's reproductive tract, as in birds. Both direct (without interaction with ovarian hormones such as estradiol) and indirect input from the components of the hypothalamo-hypophysial-ovarian and adrenal axes, control the oviductal development causing seasonal changes during the reproductive cycle such as increasing of weight and diameter (hypertrophy). Ovarian estradiol is the main substance controlling oviductal development in preparation for gravidity or gestation. Estrogen receptors have been identified in the reptilian oviduct (Girling, 2002).

The reptilian oviduct is divided into several regions: infundibulum, uterine tube, isthmus, uterus, and vagina, although some authors differentiate more oviductal regions (Blackburn, 1998). The vagina (or cervix) is the most caudal region of the reptilian oviduct which opens directly in the common urogenital or cloacal opening (Girling, 2002). The vagina is a thick, muscular region of the oviduct, that acts as a sphincter during gravidity or gestation. It undergoes seasonal hormonal variations during the reproductive cycle (Girling, 2002). In this review, we focus on the morphofunctional organization of the vagina of *Lacerta viridis* (European green lizard), an oviparous squamate and a worldwide common species. The reproductive cycle of *Lacerta viridis*, characterized by a limited period of activity (from April to July) and a long period of stasis, causes seasonal changes of the oviduct, that are typically observed in *Lacertidae*. These seasonal changes of the oviduct occur a few weeks before the reproductive season (between April and May) (Botte, 1973). In the quiescent period, the oviduct is almost filiform and the glands atrophy (Botte, 1973; Fox, 1997).

2.6.1. Anatomy

The vaginal wall comprises a mucosal, muscular and serosal layer. In the pre-ovulatory stage (end of April and the first two weeks of May), the vagina has an ovoidal cross-section, and contains numerous longitudinal folds. These folds are often ramified reaching an appreciable height and are lined by a heavily ciliated epithelium. These folds are especially prominent in the central region of the vagina (Botte, 1973; Cuellar, 1966). The folds may reduce in height in the most caudal part, disappearing almost completely at the outlet into the cloaca. The mucosa is devoid of glands (as in human and sow) but in its epithelium, together with the ciliated elements, there are abundant mucin-secreting cells

(Botte, 1973).

The muscular layer is well developed, with muscle fibers oriented both longitudinally and transversally, separated by a thick intermuscular layer. During the quiescent state (from July to March), the diameter of the vagina decreases and the longitudinal folds become lower. Furthermore, the secreting epithelial cells are entirely devoid of secretions (Botte, 1973).

The oviductal arteries mainly originate from the dorsal aorta, posterior to the cranial mesenteric artery, with the most caudal ones arising posterior to the kidney. Upon reaching the oviduct, these arteries ramify into anterior and posterior branches that supply the oviduct (Beddard, 1904a; b).

Most venous blood from the oviduct ultimately passes into the posterior *vena cava*, Oviductal veins with the efferent renal and/or the renal portal vein, or in some cases, pass directly into the *vena cava* (Beddard, 1904a; b; Jones et al., 1983). An extensive submucosal network of lymphatic vessels has been documented in the oviducts of a few lizards (Ottaviani and Tazzi, 1977).

Oviductal development is under control of estradiol, released from the ovary. External administration of estradiol in sexually quiescent or juvenile females, causes oviductal hypertrophy and increased mucosal secretion and vascularization. Estradiol receptors have been reported in the oviduct of the oviparous lizard *Podarcis s. sicula* (Paolucci et al., 1992, Paolucci and DiFiore, 1994). In ovariectomised *P.s. sicula*, estradiol treatment increased the estradiol receptor concentration in the oviduct (Paolucci et al., 1992).

2.6.2. Innervation, neurotransmitters and neuropeptides

During the reproductive season, the lizard vagina is densely innervated. The autonomic visceral nerves arise along the length of the spinal cord and may have both sympathetic and parasympathetic components. The sympathetic nerves form a short chain of ganglia adjacent to the vertebral colums (the paravertebral chain), with long postganglionic fibers running to the viscera. The middle and more caudal preganglionic fibers (lumbosacral) innervate the urogenital system, with the main transmitter being noradrenaline which increases visceral activity (while slowing digestion). In contrast, parasympathetic stimulation is cholinergic and restore the resting status (Berger and Burnstock, 1979; Wyneken, 2007).

In the viviparous lizard, *Sceloporus jarrovi,* Rooney and co-workers (1997) described adrenergic, VIP and SP-expressing nerve fibers in the posterior part of the oviduct, identified by the authors as the uterus, innervating the muscle wall and associated with the blood vessels. However, the most posterior part of the oviduct, the vagina, was not studied by this group. These authors also found that SP was expressed in some adrenergic nerves. Stimulation of adrenergic nerves caused inhibition of oviductal contractions. In contrast, VIPergic nerve fibers were not adrenergic fibers or primary afferents, but were parasympathetic (Rooney et al., 1997). Both α- and β-adrenergic receptors were detected in the uterus of the viviparous lizard, *Liolaemus gravenhorstii,* and the oviparous, *L. tenuis tenuis*, which caused contraction and relaxation, respectively, when stimulated (Zurich et al., 1971).

In contrast to the uterus, Lamanna and co-workers (1999) observed that the full-grown vagina of the oviparous lizard, *Podarcis s. sicula*, contains more intrinsic neurons that express GAL, VIP and neuronal NOS. Isolated or grouped GAL-expressing neuronal cell bodies vary in shape and size, and lay in the circular muscle layer, in the intermuscular space and in the mucosa. GAL-expressing nerve fibers, presumably of intrinsic origin, innervate the smooth muscle layer and the mucosa, and some of these fibers have a paravascular localization. A part of them co-express VIP. A very small subpopulation of intrinsic nerve fibers express only VIP. In the quiescent oviduct, GAL-expressing nerve fibers were infrequently found in the circular muscle layer of the oviductal caudal portion (presumably the uterus-vagina region) (Lamanna et al., 1999). The expression of GAL is influenced by estrogen. In the vagina of non-reproductive females (pre-ovulatory stage), 17ß-estradiol administration induced a significant increase of the number of neurons containing GAL (Lamanna et al., 1999), as seen in mammals (Howard et al., 1997), and oviposition. Nitrergic neurons are abundant in the mature reproductive vagina and have a topographic distribution similar to that of GAL-expressing neurons (Lamanna et al., 2001). The number of nitrergic neurons increases in non-reproductive females treated with 17ß-estradiol hormone (Lamanna et al., 2001). However, whether a part of these nerve fibers has an extrinsic origin, is not known. Due to the lack of functional studies, the function of neuropeptides and neurotransmitters in the regulation of the vaginal segment may only be hypothetically assumed.

2.6.3. Vaginal microbiome

At present, not much is known about the function and diversity of the microbiome in the reptilian oviduct. One study, performed in the oviparous striped plateau lizard, *Sceloporus virgatus*, using a polymerase chain reaction procedure on DNA extracted from tissue and swab samples, showed that the most abundant family in the oviduct was *Enterobacteriaceae* that made up ± 55% of the oviductal community, which was higher than in the upper intestine and lower than in the cloaca. Other families, such as *Bacteroidaceae*, *Lachnospiraceae*, and *Tannerellaceae*, made up between 1% and 10% of the microbiome composition in the oviduct. It was demonstrated that the oviduct and the upper intestine have different microbial communities than the lower intestine and cloaca, but also have differences in major families from one another. It was suggested that the unique oviductal microbiome indicates an internal egg microbiome seeded during egg development (Bunker et al., 2021).

3. Conclusion

Data from literature, obtained in several mammalian species, including human, and in non-mammalian vertebrates, clearly showed that several neurotransmitters and neuropeptides are expressed in vaginal nerve fibers (and somata), indicating that the vagina contains specific neuronal populations involved in mediating vaginal activities. Based on these data, a tentative common anatomical picture of the vagina may be depicted. NO- and neuropeptides (VIP, PACAP, GAL)-releasing nerve fibers are found closely associated with vaginal structures, and physiological studies in different species showed similar functions for each released factor.

However, the data are incomplete. Information concerning co-expression of neuropeptides/neurotransmitters is missing in most species, and functions of neuropeptides/neurotransmitters are usually not fully revealed. Furthermore, expression changes of neuropeptides/neurotransmitters due to the different hormonally regulated physiological activities of the vagina, are not clearly documented, just like the putative presence of neuroplasticity due to various pathological conditions of the vagina.

Although, mammalian animal models are predominantly used to investigate female sexual function and dysfunction, it is clear that data collected in non-mammalian vertebrate species also contribute to reveal the role of neurotransmitters and neuropeptides in vaginal activities and pathology.

At present, the knowledge of the vaginal innervation and its role in vaginal activities and pathology, is incomplete. Preliminary studies show a reciprocal relationship between microbiota and the vaginal nervous system that needs to be extended in order to understand the effects of the vaginal microbiome, of the vaginal pathologies and of administered drugs on the neuroregulation of the vagina.

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Author statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship who are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process. She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. Anna Costagliola, Corresponding author. Giovanna Liguori Luc Van Nassauw.

Declaration of Interest

None.

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