New insights into the neural network of the nongravid uterus

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Abstract

The human uterus is exposed to epigenetic factors during maturation, which might influence its neural network. The mesh muscle is formed from the circular muscle during development and maturation, and it coordinates the longitudinal and circular muscle function. The uterus has an autonomous neural network with contractility and propagation patterns that determine its reproductive potential and health during pregnancy and delivery. Emerging knowledge on the uterine neural network and mesh muscle ultrastructure contributes to new ideas and solutions on the role of intrauterine pressure and distending fluid intravasation during hysteroscopy, and even allows for improving the operative techniques of myomectomy, adenoma cytoreductive surgery and metroplasty. Good health and well-being start from the in utero stage of life. Prenatal and antenatal care are of paramount importance to minimize the risks of malnutrition and pollutants, and foster a healthy uterus. Research regarding the neural network, function and contractility of the nongravid uterus is a new chapter in gynecology that provides significant information for a better understanding and early diagnosis and treatment of uterine pathologies and early pregnancy support.

Key words: uterus, myometrium, neurotransmitter, estrogen, progesterone

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Background

The single uterine body is shaped through fusion of the bilateral Müllerian ducts during the embryological development. The thick muscular layers of the uterus derive from mesenchymal cells, while the inner part (the junctional zone and endometrium) derives from the Müllerian duct. During maturation, the outer and inner mesenchymal layers of the body of the fetal uterus give rise to the myometrium and the endometrial stroma of the adult uterus, respectively.[1]

Konishi et al. studied the prenatal development of uterine smooth muscle using light and electron microscopy in specimens obtained from 10 human fetuses between 12- and 40-week gestation period in order to establish whether the smooth muscle of the fetal uterus develops into both smooth muscle cells and endometrial stromal cells in the mature uterus.[1] Smooth muscle differentiation takes place at 18–31 weeks of gestation, with differentiation beginning at 18 weeks and the myometrium being clearly distinguishable from the inner layer corresponding to the endometrial stroma at 31 weeks.

The myometrium has muscle layers of different orientation, the innermost muscle layer being circular and the outermost muscle layer being longitudinal. These layers contribute to peristaltic contractions while the uterus is both gravid and nongravid. The middle layer, also referred to as the mesh muscle layer, corresponds to the fusion area of the 3rd muscle layer in the Müllerian ducts. This mesh-like structure with relatively scarce muscle fibers is a vascular-enriched layer, which can be affected by sex hormones, and which contributes to the autonomic uterine contraction control.[2,3] Telocytes (TCs) in the mesh muscle express receptors for progesterone and estrogen, and respond to stimulation by steroids in order to form homo- and heterocellular junctions consisting of bundles of nerves and muscle fibers.[4] The abundance of these TCs implies that they act as local pace-making cells.[5,6] In addition, TCs contact with capillaries controls myometrial blood flow.[3,7] The mesh muscle provides the communication between the longitudinal and circular muscles.[3] These primary results should be confirmed using electrophysiological studies and calcium imaging.

In a nongravid uterus, the innermost layer of the myometrium is called the junctional zone endometrium (JZE). It is the main source of peristaltic activity across the menstrual cycle. The endometrial stem/progenitor cells lie in the basalis layer of the endometrium, next to the myometrium and JZE. Myometrial cells signal to regulate endometrial mesenchymal stem-like cells (eMSCs) with self-renewal activity influenced by cyclic estrogens and progesterone secretion.[8] The uterus derives its innervation from the lumbar plexus. The lumbar plexus is formed from L1–L4 nerve roots and gives rise to the iliohypogastric, ilioinguinal, genitofemoral, femoral, and obturator nerves. As such, these nerves are important to consider when describing the contractility, neurophysiology and possible pain pathology of the uterus.[9] Endometrial waves are initiated in the sub-endometrial myometrium, but the link between the innervation of the uterus and its contractility mechanisms remain unclear. Estrogens affect the central nervous system (CNS) and, together with progesterone, play a role in neurotransmission in the uterus by acting as neuromodulators. Estrogens accelerate the frequency and intensity of uterine contractions, while progesterone decelerates them.[10,11]

Objectives

In this literature review, we analyzed and indicated the importance of available information on the uterine neu-ral network, and its anatomical localization of the uterus, within the uterus. The relation of uterine neurophysiology to uterine contractility, and the hormonal control of neurotransmission is also discussed. The presented data enable a better understanding of how: a) the uterus responds to menstrual cycle; b) pain emerges from uterus; c) myometrium compensates intrauterine pressure during a hysteroscopic procedure; d) to diminish complications due to prolonged hysteroscopic surgery, and; e) to further improve hysteroscopic surgery and its outcomes.

Materials and methods

The initial search for the data collected for this review was conducted in March 2020. The search strategy involved searching the National Center for Biotechnology Information (NCBI) and PubMed databases using the keywords 'uterus', 'uterine innervation', 'sensory nerve

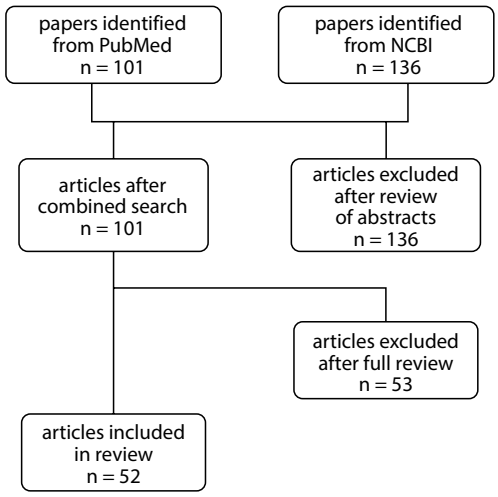

Fig. 1. Diagrammatic presentation of the methodology used for the collection of articles, and exclusion and inclusion process of the relevant papers

NCBI – National Center for Biotechnology Information.

fibers', and 'neural transmitters in the endometrium and myometrium', which revealed 101 papers. During the 2nd round of data collection, more precise keywords were used: 'endometrium', 'myometrium', 'uterine', 'adrenergic receptors', 'purinergic receptors', 'estrogen receptor (ER)', 'estrogen receptor-related proteins (ERRs)', 'substance P (SP)', 'protein gene product 9.5 (PGP9.5)', 'vasoactive intestinal polypeptide (VIP)', 'nerve growth factor (NGF)', 'TCs', 'tropomyosin receptor kinase A (TrkA)', and 'neurotrimin (NTM)'. This more focused search yielded 237 articles. Papers were selected based on their relevance to our objective, particularly excluding those that dealt with animal models or the gravid uterus, except if they held relevance to the neurophysiology and neuroanatomy of the nongravid human uterus. Figure 1 summarizes the procedure followed during data collection.

Results

Uterine innervation

Two main components form the uterine innervation, i.e., the efferent (autonomic) and afferent (interoceptive) nerves. Normal myometrium consists of autonomic innervation with TCs, which are long, thin interstitial cells with long extensions, called telopodes. The presence of extracellular vesicles along the telopodes suggests active intercellular signalling.[4] Additionally, uterine TCs express progesterone and ERs. Since estrogen influences the development of uterine innervation, it has been suggested to affect the regulation of uterine innervation and contractility.[12] Furthermore, nitric oxide-synthesizing nerves are abundant in the uterus, and can be sensory or autonomic. Neurotransmitters are secreted into the perifascicular space, and their axonal ends are not in close contact with the myocytes. The development of the sympathetic branch of the uterine autonomic nervous system can be suppressed by estrogen. The TCs express progesterone and ERs specific to their location.[13] Blood vessels and the innervation of the uterus are closely related. Despite this, multiple fibers are found lying freely within the connective tissue and muscles of the mesometrium, and in the myometrial layer of the uterine body. They are also found, to a lesser extent, in the endometrial layer of the uterine body.

The regional distribution of these nerve fibers as well as their type has been identified with the use of selective markers of sympathetic innervation. The density of autonomic nerve fibers is higher in the cervix and the tubal ampule than in the main tubal part, and is moderate in both the longitudinal and circular smooth muscle layers. However, within the vascular zone, this density is high and interposed amongst the 2 smooth muscle layers in the mesh muscle, as portrayed in Fig. 2.[14] Endometrial innervation is significantly less dense than the innervation of other

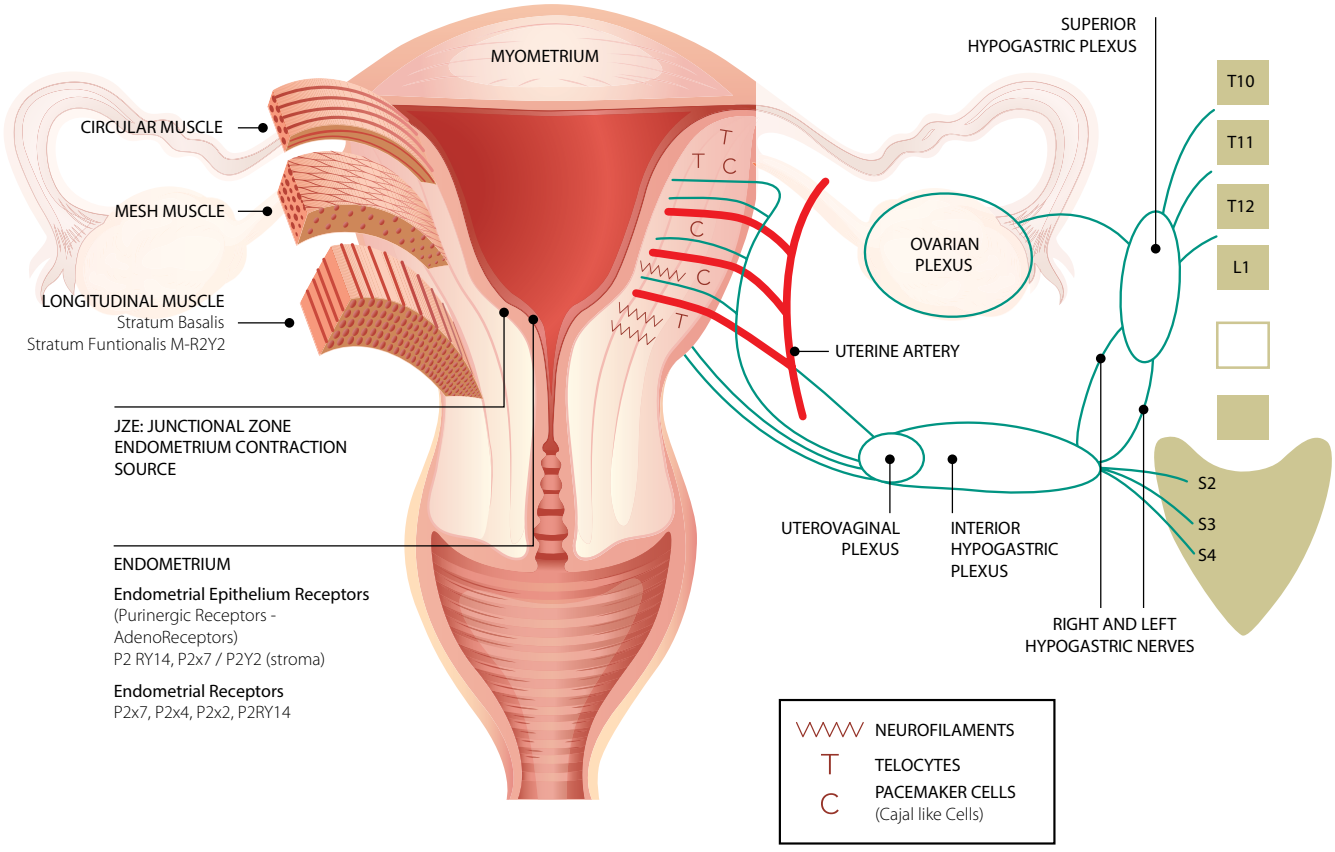

Fig. 2. Graphical representation of nerve and cell distribution

zones of the myometrium. The endometrium fibers that are present are mainly related with blood vessels of the myometrial border. This distribution may indicate that estrogens alter uterine neuritogenic properties via regulating the synthesis of brain-derived neurotrophic factor (BDNF), and thus inhibiting the outgrowth of sympathetic neurite.[15] The summary of key function cells and receptors involved in uterine neural network is presented in Table 1.

Table 1. Summary of key function cells, hormones and receptors involved in uterine neural network and contractility

References	Structure	Location	Function	Physiological response	Comments
Gibson et al. 2012[16] Bengtsson 1973[17]	ER	ERα and ERβ expressed in myometrium	ERα mRNA expression is higher than that of ERβ	estrogens produce small, frequent, local, and non-propagated contractions of the myometrium	affect development and function
Burnstock et al. 2014[18] Bottari et al. 1985[19] Hayashida et al. 1982[20]	adreno-receptor	α and β adrenergic receptors are found in myometrium using radioligand binding	regulatory mechanisms involved in contractility	α-adrenergic stimulation increases the frequency and intensity of uterine contractions, while β-adrenergic binds to epinephrine	α/β-adrenergic stimulation decreases both spontaneous and induced uterine contractility
Burnstock et al. 2014[18]	P2X1	myometrial SMC5	mediate contraction of smooth muscle	α,β-meATP induced receptor-mediated contractions associated with Cx 43	may be involved in gap junction formation
Burnstock et al. 2014[18]	P2X2/3	myometrial SMCs	mediates contraction of smooth muscle	differentiation and apoptosis of endometrial cells	changes of *P2X* R subtypes-related cells can cause differentiation and apoptosis
Burnstock et al. 2014[18]	P2X7	endometrial, endocervical and ectocervical epithelial cells	regulation of differentiation and control apoptosis	modulated vasodilatation; proapoptotic receptor plays a role in the development of endometrial cancers	these receptors shown to be immunoreactive
Chang et al. 2008[21]	P2X4	apical surface of endometrial epithelial cells	controls cervical mucous secretions	activation induces slower and prolonged junctional resistance	decreased transepithelial fluid secretion
Burnstock et al. 2014[18]	P2Y2	endometrial stromal cells	in vascular smooth muscle cause vasoconstriction, hypertension, hypertrophy	ATP acts through this receptor to inhibit cellular viability and induce early growth response 1	related to immunological impact of tissue highly occupied with neurofibers
Arase et al. 2009[22]	Ca^{++} channels	apical surface of endometrial epithelial cells	controls cervical mucous secretions	activation stimulates $[Ca2^{+}]$-dependent, chloride secretion and osmotic water efflux	increased transepithelial fluid secretion
Cretoiu et al. 2012[4] Aleksandrovych et al. 2019[12]	P2RY14	endometrial epithelial cells (+epithelial cells of cervix, fallopian tubes, placenta)	mucosal function, mediate induction of endo/trial epithelial IL-8	constant UDP-glucose expression through menstrual cycle	more abundant in endometria with PID
Bernstein et al. 2014[23]	anoctamin ANO1 ANO2	myometrial SMCs	transmembrane protein functions as Ca^{++} activated Cl^{-} channel	determine protein expression in myometrial tissue	uterine smooth muscle contractility
Wray et al. 2003[24]	Ca^{++} channels	myometrium	role in excitability and contractility	basic mechanism of uterine contractions; uncertainty which channels are present in myometrium and how action potential is initiated	unclear which channels are present in myometrium and the nature of the pacemaker mechanism
Khan et al. 2001[25]	K^{+} channels	myometrial and endometrial cells	membrane-spanning regions (M1 and M2) uterus	plays a major role in cell homeostasis and cell signaling	M1/M2 dysregulation within the intrauterine environment during adverse pregnancy outcomes
Richter et al. 2004[26]	OXTR	uterine endometrial and myometrial cells	control of cycle length, minor significance for contractility in the nonpregnant uterus	involved in parturition and lactation, increases production of prostaglandins	OXTR expression is modulated by stimulation with E_2 and OT
Cretoiu et al. 2012[4] Aleksandrovych et al. 2019[12] Varga et al. 2018[27]	TCs	interstitial cells found in all sublayers of myometrium	extracellular vesicles along the telopodes	active intercellular signaling, express ER and PR, estrogens influence the development of uterine innervation	telocytes regulate uterine SMC expression; regulating uterine innervation and contractility

Table 1. Summary of key function cells, hormones and receptors involved in uterine neural network and contractility – cont.

References	Structure	Location	Function	Physiological response	Comments
Popescu et al. 2007[28]	pacemaker cells (Cajal)	triangular-shaped cells located throughout the myometrium on the borders of smooth muscle bundles	myogenic regulation in uterus and fallopian tubes	CLC might have other physiological roles, depending on tissue type (e.g., intercellular signaling, immune surveillance, steroid sensors)	interstitial Cajal-like cells with dense cytoplasm, and numerous mitochondria
Zefferino et al. 2019[29]	GJ	myometrium cell–cell contacts formed by Cx	large number of GJs rapidly formed prior to and during parturition	rate of gap junction formation also affected by estrogen/progesterone	major mechanism in parturition

ER – estrogen receptor; PR – progesterone receptors; GJ – gap junction; Cx – connexin; TCs – telocytes; ATP – adenosine triphosphate; OT – oxytocin; OXTR – oxytocin receptor; IL-8 – interleukin 8; SMC – smooth muscle cell; UDP – uridine diphosphate glucose; PR – progesterone receptor; GJ – gap junction; ANO – anoctamin; PID – pelvic inflammatory disease; SMC – smooth muscle cell; NF – neurofilament; PGP – protein gene product; PGE2 – postaglandin E2; PGF2a – postaglandin F2a; P2RY – purinergic receptor; CLC – Cajal-like cells.

Sensory nerve fibers and neural transmitters in the endometrium and myometrium

Table 2 summarizes identifiable sensory nerve fibers in the endometrial functional layer. Following immunohistochemical analysis, several neurotransmitters and signaling proteins, including VIP, SP, neurofilament (NF), PGP9.5, neuropeptide Y (NPY), and calcitonin gene-related protein (CGRP), were examined.[36] Nerve fibers reactive to neurotensin (NT), NPY, VIP, and SP are found in normal human endometrium,[37] although little is known about their function in the uterus. In the healthy human endometrium, immunoreactive nerve fibers are restricted to the basal layer and the functional layer of the endometrium is devoid of innervation.

Neuromodulation of the uterus

Neurotrimin (NTM) is a glycophatidylinositol. This neuronal cell adhesion molecule belongs to the immunoglob-

Table 2. Reported nerve fibers and neurotransmitters found in the uterus

References	Structure	Location	Function	Physiological response	Associated pathologies	Comments
Nerve fibers						
Zhang et al. 2010[30]	PGP9.5 immunoreactive nerve fibers	basal layer of endometrium, myometrium	PGP9.5 neuronal marker for autonomic and sensory nerve fibers	found in myometrium, occasionally present in endometrium	in the endometrium of women with pain	their presence is related to the absence of pain
Zhang et al. 2010[30]	NF immunereactive nerve fibers	basal layer of endometrium, myometrium	NF – neurospecific cellular component	present only in myometrium	infiltration of endometrium cause pain	nerve fibers are related to pain
Taneike et al. 1999[31]	cholinergic nerve fibers	abundant in cervix uteri, uncommon in uterine body	inner circular muscle layer more controlled by cholinergic innervation	associated with the uterine arteries and myometrial smooth muscles	used to control blood flow to the uterine region with medication	–
Stjernholm et al. 1998[32]	small unmyelinated nerve fibers	functional layer of the endometrium	controlled by adrenergic and postganglionic fibres of the sympathetic nerves	involved in pain, thermoregulation, mechanoreceptors	involved in endometriosis	in chronic deep pain, temperature receptors, pressure or mechanoreceptors
Tokushige et al. 2006[33]	cytokines	stroma of endometrial cells	IL-8	endometrial glandular cells	present in endometriosis, absent in women without endometriosis	present in functional endometrium
Neurotransmitters						
Egarter et al. 1992[34], Mueller et al. 2006[35]	PGE_2, $PGF_{2\alpha}$	endometrium, myometrium	*PGE2* and *PGF2α* contract the myometrium by acting as a calcium ionophores	direct stimulation of myometrial contractions	inflammatory cytokine	promotes neutrophil chemotaxis
Arase et al. 2009[22]	PGE_1	myometrial SMCs	–	increase in uterine contractility	estrogen increases the production of $PGF_{2\alpha}$ by the decidua	–

IL-8 – interleukin 8; NF – neurofilament; PGP9.5 – protein gene product 9.5; PGE – prostaglandin E; $PGF_{2\alpha}$ – prostaglandin $F_{2\alpha}$; SMC – smooth muscle cell.

ulin-like cell adhesion molecule protein family. It is found in the uterus and is regulated by estrogen. Neurotrimin regulates the development and outgrowth of neuronal projections (neurites). Its expression is increased in patients with leiomyoma and in in vitro models after estrogen and progesterone treatment. In human, the *NTM* gene is situated on chromosome 11q25 and encodes a 39-kDa protein.[38] The BDNF belongs to the neurotrophin family and exerts its physiological functions through the neurotrophic tyrosine receptor kinase type 2 (Ntrk2) receptor, which is expressed in the luminal and glandular epithelium, as well as in the myometrium and vascular smooth muscle. The BDNF and Ntrk2 have an autonomous expression in the uterus.[39] The ligand and receptor are both co-localized and co-expressed in the uterus. The BDNF–Ntrk2 binding activates the adhesion formation, apoptosis, angiogenesis, and proliferation pathways, which are prominently involved in the physiology of reproduction.[40] Some transient receptor potential vanilloid type-1 (TRPV1)-immunoreactive fibers are found in nerve fascicles in the body of the nongravid uterus, but not of the gravid uterus. These fibers are sparsely distributed around the blood vessels and throughout the stroma of the cervix, but occur more frequently in the subepithelium.[40]

Purinergic receptors are classified as either P1 or P2. The P2 purinergic receptors – P2X17, P2Y1, 2, 4, 6, 11, 12, 13, and 14, have been identified using molecular cloning.[41–43] Burnstock demonstrated high affinity for extracellular adenosine in P1 receptors, while P2 receptors had high affinity for adenosine triphosphate (ATP).[42] The P2U/P2Y2 purinergic receptor has demonstrated an equal or greater response to uridine-5′-triphosphate (UTP) compared to ATP. The P2U/P2Y2 receptor mRNA is expressed in human endometrial stromal cells (hESCs). Adenosine triphosphate activates the extracellular signal-regulated kinases 1/2 (ERK1/2), inducing the phosphorylated ERKs nuclear translocation, while simultaneously increasing matrix metalloproteinase (MMP)-2, -3, -10, and -24 expression in hESCs.[21] The P2X7 receptors are also expressed in human endometrial epithelial cells. Throughout the endometrial cycle, endometrial differentiation and apoptosis are mediated by changes in the P2X receptor subtypes expression in uterine epithelial cells.[18] The P2X receptors are known to exist on sensory nerves,[44] and have been identified on uterine and cervical sensory nerves. The G-protein-coupled estrogen receptor 1 (GPER-1) mRNA, which might play a role in endometrial biology, was found in all endometrial and decidual tissue samples.[5,45,46]

Interactions between hormones and neurotransmitters involved in uterine innervation

The afferent innervation of the myometrium is immunoreactive for multiple co-transmitters, including SP, CGRP, VIP, nitric oxide synthase (NOS), neurokinin A, galanin, and secretoneurin.[47] Estrogen incites a surge in protein production, which negatively affects sympathetic nerves. Semaphorin 3F, BDNF, *NTM*, as well as substrate-bound signals and pro-NGF all contribute to remodeling of the uterine innervation, which is induced by estrogen. Pro-neuritogenic factors such as NGF and NT3 remain undiminished, while estrogen elicits alterations in favor of p75NTR and TrkA neurotrophin receptors of sympathetic neurons.[48] Estrogen in the dorsal root ganglion (DRG) sensory neurons of the uterus supply causes changes in TrkA levels,[49] which in turn affect neuronal receptivity to the pro-neuritogenic effects of NGF and NT3, while promoting BDNF inhibition.[50] The TrkA signaling in sympathetic neurons, which is stimulated by NGF, is antagonized by Sema3F.[51] The p75NTR is bound to neurotrophins in uterine-projecting sympathetic neurons required for growth by the inhibition of sympathetic neuron responses to Sema3F.[52] The p75NTR expression is increased by estrogen. Cajal-like interstitial cells have been described outside the gastrointestinal tract, including in the uterus,[53] which further contributes to spontaneous uterine contractions. The complexities of myometrial innervation remain unclear, and the impact of neuromodulation on uterine contractility is still under investigation.

Discussion

The uterovaginal plexus gives rise to the uterine sympathetic nerve fibers. The biggest component of the uterovaginal plexus is the inferior hypogastric plexus and more specifically, its anterior and intermediate components. Furthermore, the pelvic splanchnic nerve roots S2–S4 give rise to the parasympathetic fibers of the uterus. Premature labor[54] is a complication of poor uterine adaptation during fetal growth, and pathologies such as adenomyosis,[55] endometriosis,[10,55] dysmenorrhea,[10] as well as infertility[56] are complications that arise after irregular myometrial or peristaltic contractions. However, the functional mechanisms of these muscles are still unclear.

Embryological development of myometrium

The organogenesis and maturation of the human uterus take longer than in other vital organs. The myometrial smooth muscle differentiation initiates after 18 weeks of gestation and the outer myometrial layer matures after 31 weeks of gestation. This stage is also characterized by increased activity of granular endoplasmic reticulum and Golgi apparatus, which suggests early protein production. The mesh muscle identified among the longitudinal and circular muscle layers anatomically blends the 2 layers together and coordinates the uterine contractions. Kuijsters et al. based this hypothesis on the embryological merging of the 2 Müllerian ducts and the development

of mesh-like structures.[7] Endometrial cancer is considered to be in an advanced stage when malignant cells are found beyond the 50% of the myometrium, i.e., over the borders of the mesh muscle. At this point, distant metastasis and poor prognosis are expected.

Vascularization

Neural pathways follow the arterial supply. The anthropoid uterus is supplied with blood vessels from the ovarian, uterine and vaginal arteries.[57] The circumferential arcuate arteries are formed from the uterine arteries which penetrate the myometrium bilaterally.[58] This allows for a uniform capillary distribution and creation of a gradient of decreasing vascular smooth muscle richness from the exterior to the innermost layer of the myometrium.[58] Considering that the vascular layer of the myometrium is controlled by the autonomic nervous system, a correlation can be made. According to histological studies, a heavy plexus of nerve fibers with vasomotor function was identified medial to the main uterine artery, but no such structure was identified on the secondary uterine or the ovarian-uterine arteries.[59]

Localization of nerve fibers

Neurofilament and PGP9.5 are pan-neuronal cytoplasmic proteins that are used as markers for unmyelinated and myelinated autonomic and sensory nerves.[30] Nerve fibers immunoreactive with NF and PGP9.5 are mostly restricted to the myometrium, with minimal presence in the endometrial basal layer. The endometrium is largely devoid of innervation. The inner circular layer of the myometrium is mostly cholinergic, while the outer longitudinal layer is mostly adrenergic.[31] Cholinergic nerve fibers are abundant in the cervix uteri, but uncommon in the body of the uterus.[32] Additionally, in patients with endometriosis, NF-immunoreactive myelinated fibers were found in the deeper portion of the basal endometrium. In contrast, patients who did not have endometriosis had myelinated nerve fibers – except for 1 patient who did not have endometriosis, but her symptomatology was consistent with that of endometriosis (i.e., uterine fibroids, menorrhagia, pelvic pain, and chronic cervical inflammation).

Interestingly, in the functional endometrial layer, there were no NF-immunoreactive myelinated nerve fibers. This was true both for patients with and without endometriosis. Occasionally, small unmyelinated nerve fibers were noted in the deeper basal layer in women without endometriosis. Tokushige et al. identified 31 women without endometriosis who had no nerve fibers in the basal endometrial layer.[33] Furthermore, there were 4 women without endometriosis who presented with deeper basal endometrial nerve fibers, but were also diagnosed with uterine fibroids. Multiple small unmyelinated nerve fibers marked for PGP9.5 are present in the functional endometrial layer of all patients with endometriosis. However, in patients without endometriosis, no nerve fibers were detected in the endometrial functional layer.[6] The PGP 9.5 is used in the primary identification of both unmyelinated and myelinated uterine nerve fibers. Patients with PGP 9.5-immunoactive nerve fibers in the endometrial functional layer experience pain. However, patients who do not have PGP 9.5-immunoactive nerve fibers do not suffer from pain. Consequently, PGP 9.5-immunoreactive nerves can be part of a pain mechanism in gynecological conditions such as adenomyosis. Most α1-adrenergic receptors in the uterus are found in spherical bundles of the isthmo-cervical area. In contrast, β2-adrenergic receptors are mostly found in the uterine body. Tyrosine hydroxylase immunoreactivity was used to demonstrate the adrenergic contribution to the uterine innervation in myometrial tissue. The α-adrenergic receptors play a role in contraction, whereas β2-adrenergic receptors are involved in tocolysis. However, the clear roles of β1-, β3- and α2-receptors in the myometrial contractility remain incompletely understood.

Different neuronal factors can be used to create a picture of normal uterine sensory and motor function. For example, sensory function in the nongravid uterus can be estimated through the investigation of NTM, a fixed neuronal cell adhesion molecule that regulates the development of neuronal projections in the uterus, and is in turn regulated by estrogen.[5] The *NTM* expression is increased in painful uterine diseases such as leiomyoma, suggesting its role in transmitting sensory information in normal uterine neurophysiology.[38]

Chemical factors affecting myometrial contractility

A prominent inward (depolarizing) current is activated by Ca^{2+} entry into cells, and this process plays a functionally important role in myometrial contractility by contributing to membrane potential and firing frequency in myometrial cells. Other chemicals that influence contractility are hormones affecting both estrogen receptor proteins and progesterone receptor proteins. Estrogen and progesterone play a role as neuromodulators and have a hormonal effect on neurotransmission.

Hormonal control

Estrogen also contributes to an increased uterine contractility in indirect ways. Egarter and Husslein showed that estrogen leads to an increased production of prostaglandins (e.g., PGE_1, PGE_2, PGF_2) from endometrial decidua.[34] Prostaglandins also increase the contractility of the uterus.[35] Prostaglandins act as neurotransmitters (Table 2). The female genital ducts, in contrast to the male ducts, which are controlled by testicular androgens,[60] differentiate autonomously without the need for any external regulatory factors.[61] Estrogen and progesterone surge in the plasma

of both the mother and fetus during gestation. The decrease in size of the uterus following oophorectomy may negatively affect female genitalia development.[62] Hence, during pregnancy, the fetal uterus is likely influenced by both estrogen and progesterone, particularly its mesenchymal components, i.e., smooth muscle and endometrial stroma. Further research is needed to verify the relationship between sex steroids and fetal uterus development.

Limitations

The limitations of our review were largely due to the limited number of studies that met the criteria for inclusion. The scarcity of suitable studies can be attributed to the limitations of the research design that would be necessary in order to collect this data. It is logistically and ethically difficult to carry out the necessary research aimed at identifying the neural network of the living, nongravid, human uterus at the ideal level of detail and quality.

Conclusions

The organogenesis and maturation of the human uterus takes longer to occur than in other vital organs. Signs of smooth muscle differentiation are observed as early as 18 weeks of gestation. However, maturation and increase in organelles in the outer layer of myometrium, which suggests early protein production, is not observed prior to 31 weeks of gestation. The newborn uterus continues to develop during childhood and teenage until reaching adulthood, changing its dimensions in favor of the corpus, while the cervical length decreases. Given the later development of the uterus, it can be speculated that there is an increased risk of myometrium microstructure malformations and malfunctions due to the influence of epigenetic factors (e.g., malnutrition, stress, hormonal levels, pollutants, and hypoxia). Hence, each uterus reaches maturity after exposure in utero, during labor and delivery, and later in life. The organ adapts to the conditions of every stage and forms a unique complex of neural network elements and microstructure, especially in the area of the mesh muscle. These findings reflect the development of an autonomous neural network of the uterus, and the subsequent contractility and propagation patterns that affect uterine health and potential to reproduction, pregnancy and delivery. Further knowledge on uterine neural networks and mesh muscle ultrastructure may lead to new ideas and solutions regarding intrauterine pressure and distending fluid intravasation during hysteroscopy, as well as to the improvement of the operative techniques of myomectomy, adenoma cytoreductive surgery and metroplasty. Prenatal and antenatal care are of paramount importance to minimize the risks associated with malnutrition and exposure to pollutants. Studies examining the neural network, function and contractility of the nongravid uterus represent a new benchmark in gynecology research, providing significant information for better understanding and early diagnosis and treatment of uterine pathologies.

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