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The Role of Pelvic and Perineal Muscles in Reproductive and Excretory Functions

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1. Introduction

Excretory and reproductive functions are underlaid by autonomic- and somatic-neural control that regulates the pelvic and the perineal structures in mammals. Viscera and striated and smooth muscles are involved in complex and multiple reflexes occurring in the pelvic cavity (Komisaruk and Sansone, 2003; Pacheco et al., 1989; Thor and de Groat, 2010). In women, this region must accommodate the growing fetus during pregnancy and the passage of the newborn during childbirth (Ashton-Miller & DeLancey, 2007). For this to occur, the fetal head has to undergo a series of internal translations and rotations, aided by the maternal effort in the form of active pushing and uterine contraction (Li et al., 2010). Functions, such as urination and defecation, are also regulated by the different autonomic and somatic reflexes of the pelvic cavity.

During pregnancy and parturition, the fetal weight, translations, and rotations on pelvic structures, such as pelvic and perineal floor muscles, cause injury to the components of the pelvic reflexes (Kearney, 2006; Lanzarone & Dietz, 2008). This has been associated with visceral disorders, such as pelvic-organ prolapse and urinary and fecal incontinence (Ashton-Miller & DeLancey, 2007; Smith et al., 1989).

One of the techniques frequently used to evaluate the participation of pelvic and perineal muscles during normal and pathological functions is electromyography. The electromyographic (EMG) recordings are extremely useful to evaluate the participation of pelvic and perineal muscles during the normal and pathological functions of this female anatomy. The goal of our chapter is to review the literature of the activity of pelvic and perineal muscles and the several viscerosomatic reflexes involved in sexual response and urination in female mammals. Measurements of the pelvic- and perineal-muscle EMGs in laboratory rabbits, focusing on methods and results, and the alterations of their activity associated with dysfunctions, particularly with urinary incontinence, will be also reviewed.

1.1 Muscular reflex activity during parturition in women

Parturition is the physiological process of expelling the fetus from the uterus through uterine contractions, accompanied by cervical effacement and dilation. This process occurs in pregnant women at term and is divided into three stages; the first stage is the onset of effective uterine contractions until full dilatation of the cervix, the second stage is from full dilatation of the cervix until birth of the baby, and the third stage is from birth of the baby until delivery of the placenta.

The first stage of parturition begins with changes in the hormonal levels (and their receptors) of the mother, such as withrawal of progesterone and increasing levels of estrogen, oxytocin, prostaglandins, corticotrophin-releasing hormone, and cortisol, which starts the uterine contractions (Kamel, 2010). These contractions stimulate uterine mechanoreceptors that increase the firing rate of the hypogastric afferents and activate neurons at the thoraciclumbar level (Tong et al., 2003). Uterine stimulation also produces the uterocervical inhibitory reflex that decreases cervical pressure (Shafik, 1994).

The second stage of parturition is characterized by viscerovisceral and viscerosomatomotor reflexes occurring to support fetus expulsion, as the fetus moves downward through the birth canal and distends the perineum. Once the cervix is full dilated, the fetus-caused cervical distention activates the cervix-uterine reflex that increases uterine contraction and pressure, a positive uterine contraction feedback that leads to displacement of the fetus into the cervix (Shafik, 1994). Once the head has reached the base of the pelvis the mother experiences a strong reflexive desire to push. This represents a pushing, reflex reaction triggered by mechanoreceptors activated by cervical dilation and the downward progress of the fetal head, with the abdominal musculature as an effector. Relying on the entirely natural pushing reflex, instead of starting their pushing voluntarily at an early state, can further help mothers adopt the most effective pushing rhythm to reduce the time of fetus expulsion and postpartum fatigue (Yildirim & Beji, 2008).

Fetus expulsion requires a reduced resistance in the birth canal. Contraction of the musculature adjacent to the vagina may increase the vaginal resistance and push back on the fetal head, delaying the progress of the fetus. The pelvic floor muscle is a fanlike layering of striated muscle that encircles and supports the pelvic viscera (Lanzarone & Dietz, 2008; Li et al., 2010). It is named the *levator ani* (LA) and is made up of the pubococcygeus (Pcm), the puborectalis, and the iliococcygeus (Icm) muscles. Despite its close anatomical relation to the birth canal, there is little published work on the interaction between the pelvic floor and parturition (Lanzarone & Dietz, 2008). During this period, vaginocervical mechanoreceptors may produce an inhibitory reflex to turn off the LA motoneurons, located at the lumbar spinal-cord segments, to relax this striated musculature so that the fetus may pass through the pelvic cavity. The afferent axons may travel through the hypogastric nerve. When the fetus reaches the vagina and perineum, the birth canal mechanoreceptors activate the vaginocavernosus reflex, which then activates the ischiocavernosus and bulbospongiosus muscles. Contraction of both muscles forms a step to prevent the fetal head from sliding quickly from the cervix to the vaginal outlet and prevent tearing (Shafik, 1993).

The third stage of parturition starts once the baby has been expelled. Delivery does not end until the placenta and membranes are shed. This occurs in response to a continuous uterine contraction that produces uterine involution, which favors the detachment of the placenta. Once the placenta is completely detached from the uterine wall, a woman may feel contractions again and want to push to produce what is called birth or delivery of the placenta and membranes (Kamel, 2010).

1.2 Micturition and pelvic floor muscle activity in women

Micturition in mammals is a vital physiological process involving two coordinated events; the storage and expulsion of urine. Both events have as substrate the lower urinary tract (LUT) and are regulated by autonomic (hypogastric nerve consisting predominantly of sympathetic, and pelvic nerve, mainly parasympathetic) and somatic (represented by the pudendal and LA nerve) components triggering spinal and supraspinal reflexes to coordinate the activity between the LUT components (urinary bladder, urethra) and the striated musculature (Barber et al., 2002; Fowler et al., 2008).

The role of the striated muscles in micturition has been discussed and has been a controversial subject for several years (Bors and Blinn,1964; Lapides et al., 1957; Shafik, 2003; Yang & Huang, 2002; Thor & de Groat, 2010). To date the importance of the striated muscles to maintain normal function in both continence and urine release is recognized.

During urine storage the bladder is inactive, but the internal and external sphincter show tonic contraction (Shefchyk, 2002.) that helps to support urinary continence. This occurs when the afferents of the hypogastric nerve inhibit the bladder parasympathetic-preganglionic neurons at the 2-4 sacral spinal segments and activate the Onuf's nucleus motoneurons to contract the external urethral sphincter, causing urethral closure and preventing involuntary urine release. This process is organized by urethral reflexes known collectively as the 'guarding reflex' (Fowler et al., 2008). Whereas the contraction of the smooth muscle of the bladder neck and urethra are regulated by the hypogastric nerve (Andersson & Waldeck, 2001), other nonneural elements that contribute to the urine storage and urethral closure are vascular elements of the urethra, such as the arteriovenous plexus (Ashton-Miller & DeLancey, 2007). Through the regulation of supraspinal sites, pelvic floor muscles can also be voluntarily contracted to maintain urinary continence (Fowler et al., 2008).

During voiding, sphincter activity stops as the bladder contracts so that voiding is efficient (Yalla & Resnick**,** 1997). When the bladder reaches its threshold, the bladder-wall mechanoreceptors activate myelinated afferents (Aδ) and the unmyelinated or the C-fibers of the pelvic nerve. The afferents send information to the spinal cord (Fowler et al., 2008) and the ascending pathways to the brain stem, specifically to the pontine center. This integrates information from the spinal cord and brain regions, such as the hypothalamus and the cortex, acting as a micturition-reflex switch. The central pontine sends information to the 10-2 toraculumbar level to inhibit the sympathetic neurons and produce relaxation of the smooth muscle of the bladder neck and urethra, whereas at the 2-4 sacro level it inhibits the motor neurons of the pudendal and LA nerves to produce relaxation of the external urethral sphincter and pelvic floor muscles, allowing the descent of the bladder neck and the opening of the urethral lumen. At that level the parasympathetic-preganglionic neurons of the pelvic nerve are excited (Shefchyk, 2001) to trigger the detrusor contraction (Andersson & Waldeck, 2001; Morrison, 1999).

Thus, urine output requires the contraction of the detrusor muscle in coordination with the relaxation of the smooth muscle of the bladder neck, the external urethral sphincter, and pelvic floor muscles. In contrast to the pelvic muscles, activation of the abdominal and perineal-striated muscle facilitates urine expulsion during normal voiding (Shafik**,** 2003). Thus, the passage of urine through the urethra stimulates mechanoreceptors located in the urethral wall to produce the reflex contraction of the perineal muscles, called the urethra-corporocavernosal reflex (Shafik et al., 2008).

During the vaginal delivery, in the second stage of labor, the fetus puts pressure on the LUT and the LA, which can cause damage to these structures, nerve injury, and loss of connective

tissue (Abdool et al., 2009; Branham et al., 2007; Dietz et al., 2008; South et al., 2009). The failure to recover from this trauma may lead to permanent neuromuscular, pelvic floor, and micturition disorders. These are important factors in the development of pathologies, such as pelvic-organ prolapse and fecal and urinary incontinence in parous women, which is a common medical condition.

Animal models have aid our understanding of the mechanisms underlying the pelvic visceral function and dysfunction.

2. Animal models for studying the physiology of pelvic and perineal muscles

2.1 The laboratory rat

The rat has been the animal model commonly used to study the mechanisms of urogenital function because of its maintenance cost and availability. Viscerosomatic and somatosomatic reflexes activated during mating and parturition have been well-documented in this species (Brink et al., 1980; Cueva-Rolon et al., 1996; Martínez-Gómez et al., 1992; Pacheco et al., 1989). Stimulation of mechanoreceptors located in the perineal skin and distal vagina activates pelvic floor muscles, such as the Pcm and the Icm. The Pcm and the Icm originate from the medial face of the pelvis and insert onto the proximal tail (Brink & Pfaff, 1980). Their fibers close the pelvic outlet cavity and are in close anatomical relation to the vaginal tract and rectum. The Pcm and the Icm together are part of the LA (Bremer et al., 2003). However this name may be confusing because a sexually dimorphic muscle, only present in male rats, has received the same name (Cihak et al., 1967).

Because of their anatomical position and their reflex activity, the Pcm and the Icm have been involved in sexual, reproductive, urinary, and defecator functions. In anesthetized rats the Pcm and the Icm muscles contract in response to mechanical stimulation of the perigenital skin and vagina (Martínez-Gómez et al., 1992; Pacheco et al., 1989). These reflexes may be triggered during mating by the somatosensory stimulation that the male applies during mounts and intromissions to the female perigenital skin and vaginal tract (Pfaff et al., 1977). The reflex contraction of the Pcm and the Icm may contribute to the sexual function by supporting the posture of lordosis, a typical sexual behavior assumed by female rats. This consists of arching the back, elevating the rump, and moving the tail base to expose the vaginal orifice to the male (Pfaff et al., 1977). This proposal is supported by the finding that electrical stimulation of the Pcm and the Icm in anesthetized animals causes tail movements, vaginal orifice movements, and increases the intravaginal pressure (Martínez-Gómez et al., 1992; Poortmans & Wyndaele, 1998). Increased intravaginal pressure may increase penile stimulation to achieve male ejaculation, facilitate the retention of sperm, and enhance the quality of stimulation received by the female (Martínez-Gómez et al., 1992).

From histochemical, anatomical, and electrophysiological studies, the neural elements of the genitopubococcigeus reflex described above are sensory receptors located in the perineal skin and vaginal wall, the pudendal nerve, and the viscerocutaneous branch of the pelvic nerve and are afferent pathways. The somatomotor branch of the pelvic nerve is an efferent pathway, the L6-S1 spinal cord segment is a final common pathway, and the integrator center and pubococcygeus muscle fibers are effectors (Cuevas et al., 2006; Martínez-Gómez et al., 1992; Pacheco et al., 1989).

The role of the Pcm and the Icm in the control of female reproductive or urinary functions remains unclear. Although it has been shown that cervical stimulation inhibits the activity of pelvic floor muscles, the physiological significance of this reflex it is unknown. Denervation

of the Pcm and the Icm does not affect parturition (Martínez-Gómez et al., 1998). In addition, preclinical studies focused on the role of the pelvic musculature in micturition are scarce and results are conflicting. In male rats, activity of the Pcm occurs during micturition (Manzo et al., 1997). However, this result was not replicated in female rats (Jiang et al., 2010). Neither the Pcm nor the Icm discharged during voiding or during a manual increase of the bladder pressure, suggesting that the pelvic muscle activity is not necessary for voiding and does not contribute to the continence in the guardian reflex (Jiang et al., 2010). In another study, the Pcm and the Icm were activated during voiding only when the filling rate was high (0.5 mL/min) (Thor & de Groat, 2010). It is possible that these differences stem from variation in conditions, and more studies are necessary to unveil the function of the pelvic floor muscles on visceral functions.

Perineal muscles such as the bulbocavernosus, also called the bulboespongiosus (Bsm; Martínez-Gómez et al., 1997) and the ischiocavernosus (Ism), which are located outside of the pelvic bone, may also contribute to the visceral function. In women the bulbocavernosus and the Ism fibers are in close anatomical relation to the clitoris, vagina, and urethra (Shafik et al., 2008), and the contraction of these muscles may contribute to vaginal and urethral closure.

Unfortunately, studies to elucidate their role in the urogenital-tract function have not been possible in the female rat because the perineal-striated muscles are significantly involuted by adulthood. This requires further investigations into other potential laboratory animal models of pelvic and perineal physiology.

2.2 The domestic rabbit

The female rabbit is one alternative animal model known for its well-characterized and conspicuous pelvic and perineal musculature (Martínez-Gómez et al., 1997). The pelvic floor muscles, the coccygeus and the Pcm, and the perineal muscles, the Bsm and the Ism, are well-developed and accessible to manipulation and electromyographic recording. Therefore, the rabbit is an appropriate model to study the activity of pelvic and perineal muscles in relation to urogential functions.

The female rabbit is an induced ovulator and one intromission by the male accompanied by a brief series of pelvic thrusts is sufficient to ensure ejaculation, ovulation, and pregnancy (Ramírez & Lin Soufi, 1994; Soto et al., 1984). The female rabbit gives birth in a separate and well-disguised nursery burrow presumably to avoid predation but also because the umbilical cord usually ruptures in the long vagina, and the pups are expelled in rapid succession. In domestic breeds, the birth of as many as 14 pups is achieved within 10 – 15 min (Fuchs and Darwood, 1980; Hudson et al., 1995).

The reproductive tract of the female rabbit has two uterine horns with two necks attached to a long vagina, which we have regionalized as abdominal, pelvic, and perineal (Cruz et al., 2002). The pelvic vagina is covered by a prominent venous plexus and in its ventral wall is the urethral opening. The perineal vagina, the most caudal portion of the vagina, protrudes outside the pelvic cavity and ends at the urethra-vagina junction. Around this anatomical structure, also called the urogenital tract, the pelvic and perineal muscles are located (Fig. 1) and contribute to the reproductive and excretory functions (Martínez-Gómez et al., 1997; Cruz et al., 2002, 2010A, 2010B). The pelvic muscles include the *obturatorius internus*, the coccygeus, the Icm, and the Pcm. The perineal muscles include the *constrictor vestibuli*, the *constrictor vulvae*, the bulboglandularis, the Ism, and the Bsm.

Because of the anatomy of the urogenital tract, pelvic and perineal muscles could participate in the process of micturition and in those associated with reproduction. In rabbits, males and females have different ways to expell urine depending on the social context, which have been related to a role of chemical communication (Bell, 1980). To determine the role of those muscles in reproduction and micturition, our group has used electromyography and cystometrography (*see* below).

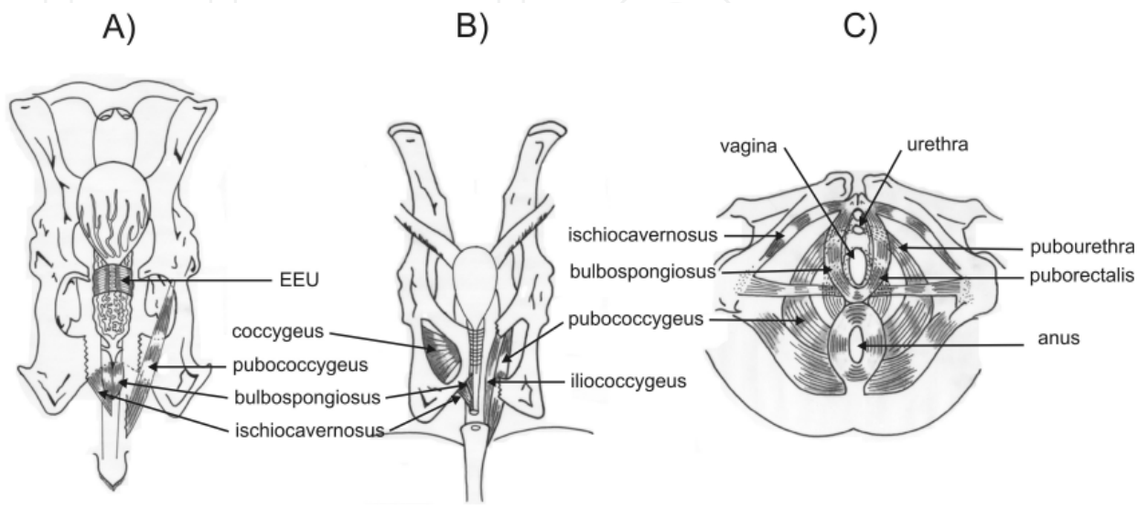

Fig. 1. Pelvic and perineal muscles in the female rabbit (A), the female rat (B) and women (C). EEU, external urethral sphincter.

3. Electromyography protocols for evaluating the role of pelvic and perineal muscles in reproduction and micturition

Electromyography methods have been widely used in research and the diagnosis of patients with neuromuscular disorders (Stalberg & Falck, 1997). The electrophysiological information is still an important part of the study of normal and abnormal nerves and muscles. In addition to morphological and histochemical methods, needle electromyography can be used to study the structure and function of the motor units (Stalberg & Falck, 1997). Electromyography has the advantage of being relatively well-tolerated, repeatable, and several muscles can be studied simultaneously. Furthermore electromyography also reflects some functional aspect of the motor units, especially those of the neuromuscular transmission.

By using electromyography in female rabbits, we have studied the activity of pelvic- and perineal-striated muscles, and some viscerosomatic reflexes, involved in the sexual response and micturition. In addition, we have evaluated how the reproductive experience affects the activity of pelvic and perineal muscles and its relationship to urodynamics.

3.1 Vaginocervical stimulation

The vaginal tract is stimulated by the intromission of the penis during mating, by the passing of the pups during parturition, and by the voiding of urine during micturition. In these processes the striated muscles associated with the vaginal tract are reflex activated, a reflex called vaginocavernosus, and this has been suggested to participate in the erection of the

clitoris and helps to prevent vaginal and perineal tearing (Shafik, 1993). In our laboratory we have evaluated the contribution of pelvic- and perineal-striated muscles to this reflex.

Stimulation of the vagina was done with a 0.5-cm diameter glass rod coated with mineral oil. This was touched to the clitoris and introduced to each level of the perineal vagina, the pelvic vagina, and the abdominal vagina, including the cervices, and then withdrawn. A possible inhibitory effect of vaginocervical stimulation on the activity of striated muscles is also analyzed. The dissection of muscles has been described elsewhere (Martínez-Gómez et al., 1997). A longitudinal incision in the skin on the right or left side of the perineum, according to the study, is made. The adipose and connective tissue, the lateral vagina, and the rectum are carefully dissected. Bipolar electrodes (0.01-mm silver wires) are inserted into the medial portion of the muscles generally, but the placement can be variable depending on the morphology and disposition of each muscle. The electrodes used for the EMG are fastened to the perigenital skin by a fine suture before inserting them into the striated muscles. The electrical activity is amplified by a preamplifier connected to a Grass PolyView Recorder 2.5 installed in a computer. Electromyographical activity is also monitored using an audio feed connected in parallel to the preamplifier.

Reflex activity in the pelvic and perineal muscles is produced by squeezing the perineal vagina between thumb and forefinger while stimulating the pelvic or abdominal vagina with the rod. In some animals, vaginal stimulation is also made after inserting a 0.7-cm diameter plastic cylinder 3-cm long into the perineal vagina, 15 min after applying a local anesthetic to the mucosa of the perineal vagina. The first method is used to prevent the rod touching the internal walls of the perineal vagina during stimulation of the pelvic or abdominal levels and the second is used to confirm that the activity of the muscles is caused by mechanoreceptors located in the walls of the perineal vagina. The activity of each muscle is recorded in each doe 2–3 min before, during, and 2–5 min after stimulation. Each stimulus is administered to the perigenital skin and at each level of the vagina at least twice, with the level and type of stimulation applied in random order. An interstimulus interval of 2–3 min is used, or if the muscle responded with afterdischarge or inhibition, an interval of 5–10 min after the after discharge or inhibition had ceased.

Another technique to measure the intravaginal pressure is the use of a balloon attached by a catheter to a pressure transducer and connected to a driver amplifier (Fig. 2). The uninflated balloon is inserted into each vaginal level and inflated via a syringe to a diameter of 2–3 cm. Then the muscles are stimulated with bipolar stainless steel electrodes using square pulses of 0.1- to 0.2-ms duration, variable voltage, and rates of 1, 5, and 10 Hz. The two distal ends of the transected nerves are bilaterally stimulated using bipolar, silver chloride electrodes connected to a stimulus isolation unit activated by a stimulator using square pulses of 0.1- to 0.2-ms duration and a variable voltage and rate.

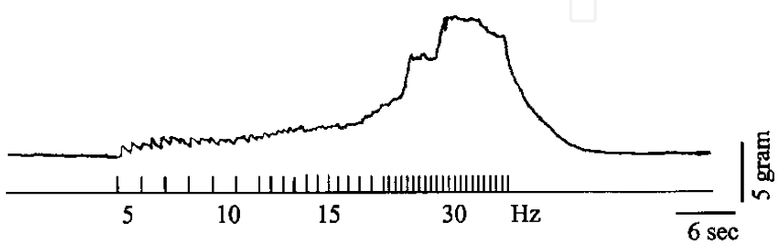

Fig. 2. Increase of the intravaginal pressure in a female rabbit during the contraction of the Bsm (Cruz et al., 2002). Physiology & Behavior by ELSEVIER INC. Reproduced with permission of Elsevier INC. in the journal format via the Copyright Clearance Center.

3.2 Simultaneous electromyography and cystometrography

Cystometry is defined as the urodynamic value that evaluates the intravesical pressure through a transducer that records the changes of pressure in the bladder during the storage and expulsion of urine. The result is a graph showing the change of bladder pressure caused by an increased volume in the bladder and these are called cystometrograms (CMGs) (McMurray et al., 2006). The recording can be made in anesthetized animals. The CMG combined with other techniques (EMG, neurectomies, or stimulation of nucleous superior centers) is used to describe the neural control of micturition, to analyze the effect of drugs and some pathologies (Pandita et al., 2000), and to evaluate the participation of pelvic- and perineal-striated muscles related to some dysfunctions.

In our work group, a CMG recordings are made using an adaptation of the previously described technique (Maggi et al. 1986). The animals are anesthetized with urethane and fixed into a dorsal supine position. The bladder is exposed through a midline incision in the abdomen and urine is expelled by applying slight manual pressure over the bladder. A 20-gauge butterfly needle is inserted into the lumen through the bladder apex to infuse warm (39 °C) saline solution (0.9% NaCl) at a constant rate of 0.8 mL per minute. The room temperature is 25 °C. Those experimental conditions were previously established at our laboratory. With polyethylene tubing (outer and inner diameter 1.6 mm and 1.2 mm) the needle is connected to a transducer to record variations in the intravesical pressure. The pressure transducer is connected to a direct current amplifier and displayed on a Grass PolyView Recorder 2.5 installed in a computer. After a 10-minute equilibration with no saline infusion, 3 reproducible bladder contractions are triggered in response to continuous saline infusion by a syringe pump.

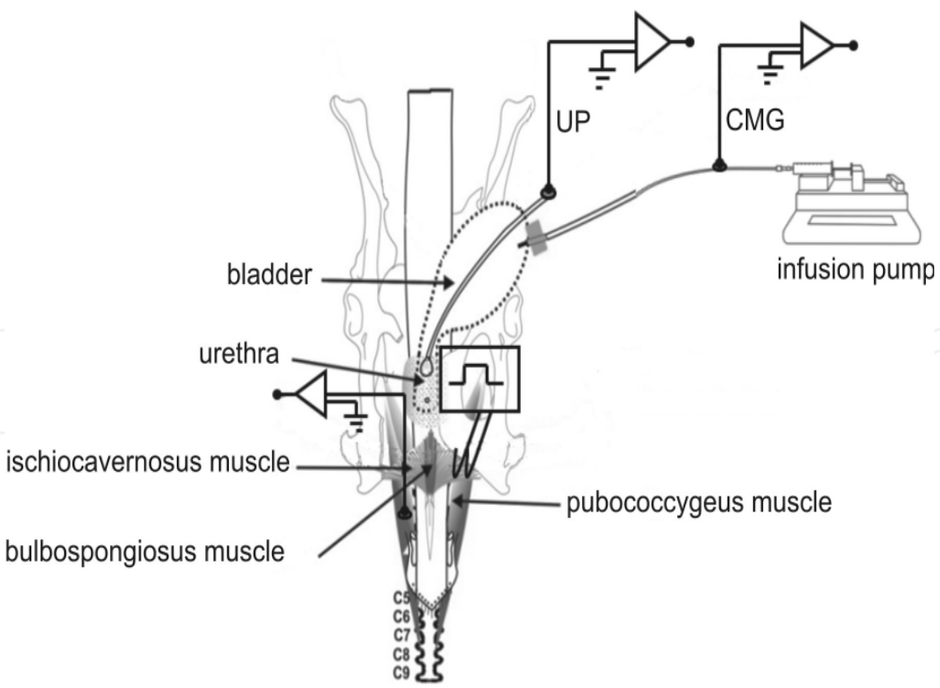

Fig. 3. Depiction showing the location of catheters used to record cystometograms (CMG) and urethral pressures (UP), and electrodes to record or stimulate pubococcygeus, ischiocavernosus, and bulspongiosus muscles in anesthetized female rabbits.

The variables analyzed to evaluate urodynamics (Fig. 4B) are 1) the volume of saline solution that triggered voiding (ThV), 2) the volume of saline expelled by the urogenital meatus during voiding (VV), 3) the volume of saline remaining inside the bladder as the result of ThV - VV (RV), 4) VE, calculated as [(VV/VV - RV) - 100], 5) the pressure that triggered the voiding phase (ThP), 6) the pressure measured from the ThP to the highest pressure observed at voiding (MP), 7) the elapsed time from the onset of ThP to the baseline pressure (VD), and 8) the elapsed time between 2 voiding phases (ICI).

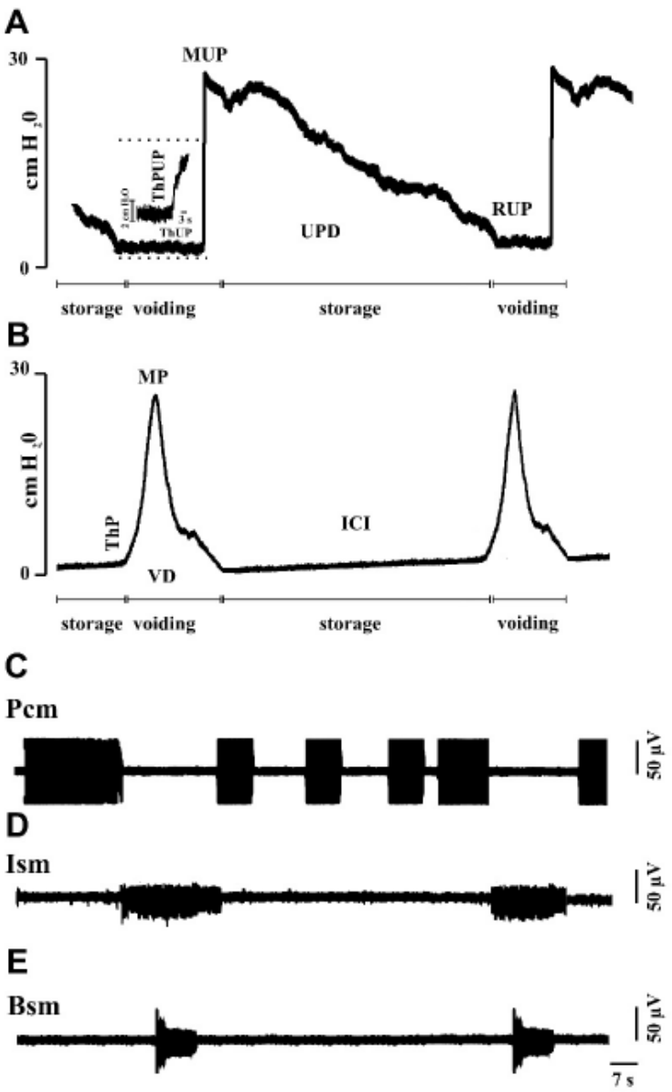

Fig. 4. Simultaneous recordings of urethral pressure (A), CMG (B), and EMG (C) of pelvic- (Pcm) and perineal- (Ism, Bsm) striated muscles during micturition caused in anesthetized female rabbits. Storage and voiding phases of the micturition are indicated. The urethral pressure is a maximum at the end of the voiding phase, preceded by the activity of the Ism and the Bsm. Thereafter, the urethral pressure decreases during the voiding phase of micturition when the activity of the Pcm occurs. s = seconds.

Electromyograms are simultaneously recorded along with the cystometograms (Fig. 3 & 4). Striated muscles (the Pcm, the Ism, or the Bsm) are dissected according to Martínez-Gómez et al. (1987) and EMG bipolar electrodes (0.01-mm silver-wires) are inserted into them. The electrical activity is amplified by a preamplifier connected to a Grass PolyView Recorder 2.5. In addition, muscle activity is monitored by using a sound unit. During the experiment, a muscle is considered to be activated or inhibited when the EMG trace is increased or decreased.

3.3 Urethral pressure

The urethral pressure (UP) is defined as the fluid pressure needed to just open a closed (collapsed) urethra (Griffiths, 1985). This definition suggests that the urethral pressure is similar to ordinary fluid pressure, i.e. it is a scalar (does not have a direction) quantity with a single value at each point along the length of the urethra. The concept of urethral pressure is only useful if the urethra collapses easily at attainable pressures to a zero cross-sectional area, as is normally the case. The use of a catheter introduces a nonzero cross-sectional area (given by the probe) and changes the natural shape of the lumen. The effect on the measured urethral pressure is small for highly distensible and collapsible tubes (Griffiths, 1985).

In our laboratory, we have established the conditions to record the urethral pressure with the EMG of pelvic and perineal muscles and the CMG, simultaneously. (Fig. 3 & 4). For this the animals are anesthetized with urethane and fixed into a dorsal supine position. For the UP recording, an adaptation of the balloon method (Lose et al., 2002) is used. A small incision is made in the bladder apex, and a balloon, mounted concentrically on a catheter (1.6-mm OD and 1.2-mm ID), is introduced into the urethra and passed though until it is exposed out of the opening of the vagina. A cotton thread (8-cm length) is tied to the balloon, which then is pulled (5 - 6 cm) inside the urethral tract. This located the balloon in the medial portion of the urethra to measure the urethral pressure. A pressure of 2-cm H_2O is required to inflate the balloon to its maximum diameter. The bladder incision is then sutured. For recording variations in the urethral pressure the catheter is connected to a transducer, which is connected to an amplifier, and the data are displayed on a Grass PolyView Recorder 2.5 installed in a computer. The urethral pressure variables analyzed are 1) the threshold urethral pressure (ThUP), defined as the pressure that triggered an abrupt rise in the urethral pressure, 2) the maximum urethral pressure (MUP), 3) the time elapsed between the ThUP and the triggering of the MUP (ThtUPD), 4) the pressure at which the UP returned to the base line (RUP), 5) the time elapsed from the ThUP to the next basal pressure (UPD), and 6) the MUP/MP ratio as an indicator of the urethral pressure required to close the urethra (UPC).

4. Reflex activity of pelvic and perineal muscles in female rabbits

4.1 Pelvic and perineal muscle activity during vaginocervical stimulation

In our laboratory, we have described the electromyographical activity of the Pcm, the Ism, and the Bsm in response to vaginal and cervical stimulation. Furthermore, mechanical stimulation, by passing a small balloon filled with saline into different regions of the vagina, differentially activated these muscles. Stimulation causes electromyographic activity of the Ism and the Bsm (Cruz et al., 2002; 2010A) and the Pcm (Cruz et al., 2010B). Such findings are similar to those described in women as the vaginocavernosus and the vaginolevator reflexes (Shafik, 1993, 1995).

In rabbits, perineal muscles such as the Bsm and the Ism are well developed, with their fibers originating at the ischiadic arch and inserting onto the ligamentum suspensorium clitoridis. Branches of the clitoral nerves innervated the BSM and the perineal nerves innervated the ISM. Bilateral electrical stimulation of these nerves caused retraction of the clitoral sheath and an increase in the intravaginal pressure at the level of the perineal vagina (Fig. 5). Though none of the muscles responded to stimulation of the perigenital skin, both were reflexively activated during mechanical stimulation of the inner walls of the perineal vagina. Prolonged cervical stimulation inhibited this reflex. Thus, in reproductive processes such as copulation and parturition, the contraction of these muscles may be produced during stimulation of the perineal vagina (Cruz et al., 2002).

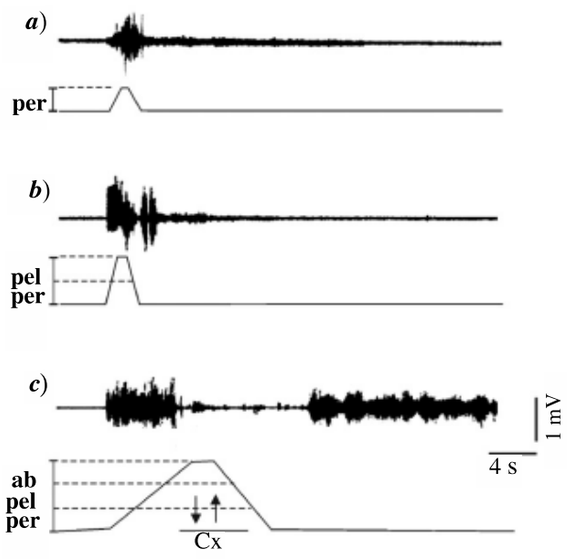

Fig. 5. Reflex EMG activity of the pubococcygeus muscle in a female rabbit in response to stimulations of the vaginal tract with a glass rod (Cruz et al., 2010b). The recording made on a polygraph before, during, and after vaginal stimulation was applied to the three levels of the vagina; a) perineal, b) pelvic, and c) abdominal. The cervix was also touched. The dotted lines shown below each EMG represent the type of stimulation. The sloping line shows when the rod was introduced, the plateau occurred when the rod reached the vaginal level (per, perineal; pel, pelvic; ab, abdominal) and the descending line shows when the rod was being withdrawn. The muscle had an afterdischarge following the removal of the glass rod.

The pelvic muscles, such as the Icm and the Pcm, are long bilateral muscles with fibers attached to the ilium bone. The muscle fibers are not attached to the pelvic viscera but they run adjacent to the vagina and rectum to be inserted into the sacral vertebrae. The iliococcygeus and pubococcygeus muscles are innervated by branches arising from S3 and S4. The reflex EMG activity was obtained during stimulation of the vaginal orifice skin and the perineal and the pelvic vagina (Fig. 6). Abdominal vaginal stimulation did not cause an EMG response. Cervical stimulation produced a temporary inhibition in the activity of both. These findings suggest that the Icm and the Pcm muscles can be activated by reflex action during reproductive processes such as mating and parturition (Cruz et al., 2010b).

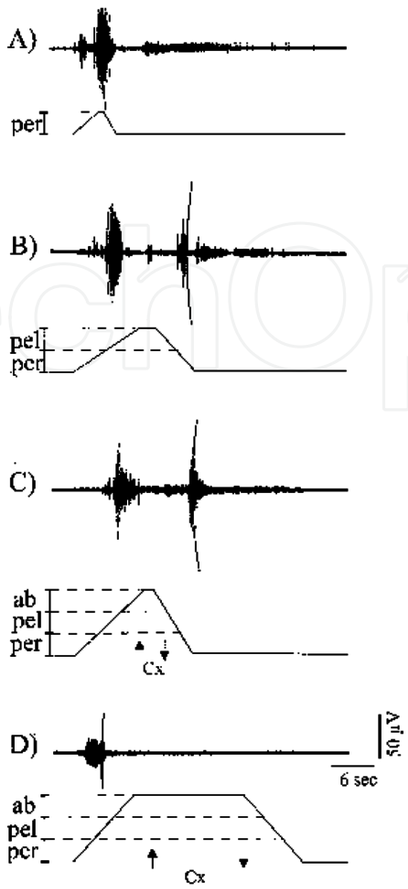

Fig. 6. Reflex EMG activity of the Bsm in a female rabbit in response to stimulation of the vaginal tract with a glass rod. Essentially, the same pattern of activation was observed in the Ism (Cruz et al., 2002). The EMGs were recorded on a polygraph before, during, and after vaginal stimulation was applied to the three levels of the vagina; (A) perineal, (B) pelvic, and (C and D) abdominal, including a brief (C) or prolonged touching of the cervix (Cx; D). The profile below each EMG shows the type of stimulation: before introducing the rod, the sloping line when the rod was introduced, the plateau when the rod reached each level of the vagina (per = perineal, pel = pelvic, ab = abdominal) and the descending line when the rod was being withdrawn. Note that except for (D), the muscle showed an afterdischarge following removal of the rod. Physiology & Behavior by ELSEVIER INC. Reproduced with permission of Elsevier INC. in the journal format via the Copyright Clearance Center.

In spite of the anatomical arrangement that the pelvic (Barber et al., 2002; Cruz et al., 2010b) and perineal muscles have in women and female rabbits (Cruz et al., 2002; Shafik et al., 2005), there are similarities of the reflex activity of those muscles. The activation of the Pcm in response to the stimulation of genital structures caused the vaginolevator reflex in women (Shafik, 1995) and female rabbits (Cruz et al., 2010b). This reflex plays a role in the sexual act that could increase the intravaginal pressure, thus increasing the stimulus received by the penis to facilitate the ejaculation (Shafik, 1995). The dysfunction of the vaginolevator reflex may result in sexual disorders (Handa et al., 2008).

Vaginal stimulation causes the reflex activity of the Bsm and the Ism (Shafik, 1993; Cruz et al., 2002; Cruz et al., 2010a), which is called the vaginocervical reflex that participates in the

erection of the clitoris (Shafik, 1993). Furthermore, the distension of the vagina by the fetal head during delivery causes the contraction of perineal muscles, which has been suggested to support the head during its descent and prevent vaginal and perineal tears (Shafik, 1993; Cruz et al., 2002; Shafik et al., 2007; Cruz et al. 2010a).

4.2 Pelvic- and perineal-muscle activities during micturition

An anatomical feature of the rabbit is that the mouth of the urethra is located in the pelvic vagina, thus forming the urogenital tract (Rodríguez-Antolín et al., 2009). Reproductive and micturition processes occur in this tract, suggesting that, as in women, pelvic and perineal muscles may be involved in the micturition process.

Micturition is a vital physiological process that involves two main phases; the continence of urine during storage in the bladder and the expelling of the urine. Because of this, in our laboratory we studied if the pelvic and perineal muscles are activated during micturition and whether the blockade of these muscles produces changes in urodynamic variables of the anesthetized rabbit (Corona-Quintanilla et al., 2009). In our study, we caused bladder emptying in young virgin rabbits by continuous infusion of saline into the bladder. During this process, the bladder pressure was measured simultaneously (CMG with the EMG of these muscles during storage and the expulsion of the saline). From our experimental observations, we established that the Pcm, the Ism, and the Bsm are active during micturition, i.e. the Pcm showed tonic activity during storage, inactivity during the voiding, and postdischarge after emptying the bladder. The Ism activity is at the end of storage and during voiding and the Bsm also showed increased EMG activity during maximum bladder pressure, corresponding to the voiding (Fig. 4B-E). In addition, another group of young virgin rabbits showed that the blockade of muscle activity by the intramuscular injection of lidocaine produces changes in the recording of the CMGs and the urodynamic variables (Corona-Quintanilla et al., 2009). The blockade of the Pcm activity, caused a significant reduction in the values of vesical efficiency and the threshold and maximum pressures. Furthermore, blocking the reflex activity of the Ism and the Bsm had a significant decrease in the vesical efficiency, intercontraction interval, and maximum pressure. With these results we concluded that pelvic and perineal muscles are differentially activated in sequence during micturition, not activated simultaneously as a unitary mass or functional unit (as seen in the literature) but as a group of structures, organized in time and space, leading us to assume the existence of a motor pattern generator in the spinal cord that generates the motion sequence for the process of micturition.

The activity of pelvic and perineal muscles during the micturition of women and female rabbits show some similarities. For example, the Pcm activity shown during the storage phase is a major component in the mechanism of urinary continence, because the electrical stimulation inhibits the bladder contraction and the urine output (Alves et al., 2011; our unpublished results). This is possible because the contraction of the Pcm during voiding is produced by the discharge of some of its afferents (from muscle spindles or tendon organs), sending information to both sacral 2 and 4 to modulate the activity of parasympathetic neurons (Manzo et al., 1997; Thor & de Groat, 2010). The Bsm and Ism activity during the voiding is mediated by a reflex called the urethrocorporocavernosal reflex. This reflex facilitates the urine voiding (Shafik et. al., 2008). Also, both women and female rabbits have been reported that during childbirth damage is produced of the pelvic floor muscles and nerves, leading to cause uncoordinated activity of pelvic and perineal muscles, modifying the micturition and producing postpartum urinary incontinence (Deindl et al., 1998; Haylen, 2009; Martínez-Gómez et al., 2011).

4.3 Pelvic and perineal muscles in virgin and multiparous rabbits

We have noted that multiparity affects pelvic and perineal muscles and this is related to the high prevalence of urinary incontinence in women with more than one vaginal delivery (DeLancey et al., 2003; Foldspang et al., 1992; Lien et al., 2004; Perucchini et al., 2002). Because of this, we studied, with the simultaneous recording of CMGs and EMGs, the Pcm, the Ism, and the Bsm. The bladder function was assessed using standard urodynamic variables. The temporal coordination of pelvic- and perineal-striated-muscle activity was changed in multiparous rabbits. The cystometrogram recordings were different from those obtained from virgin rabbits, resulting in alterations of the threshold volume, the residual volume, the voiding duration, and the maximum pressure.

Multiparity (4 consecutive vaginal deliveries) causes uncoordinated activity of pelvic and perineal muscles, and modifies urodynamics (Fig. 7; Martínez-Gómez et al., 2011). All multiparous rabbits included in that study had a pattern of muscle activity different than that of the virgin rabbits. Such a pattern involved at least one muscle in one phase of micturition. In addition, several urodynamic variables were also modified in multiparous rabbits. Those data showed that the activities of pelvic and perineal muscles are associated with the urodynamic function in rabbits. Probably the forces of the vaginal-delivery distension could injury the innervation and the morphology of those muscles, thus causing changes in their EMG activity during micturition.

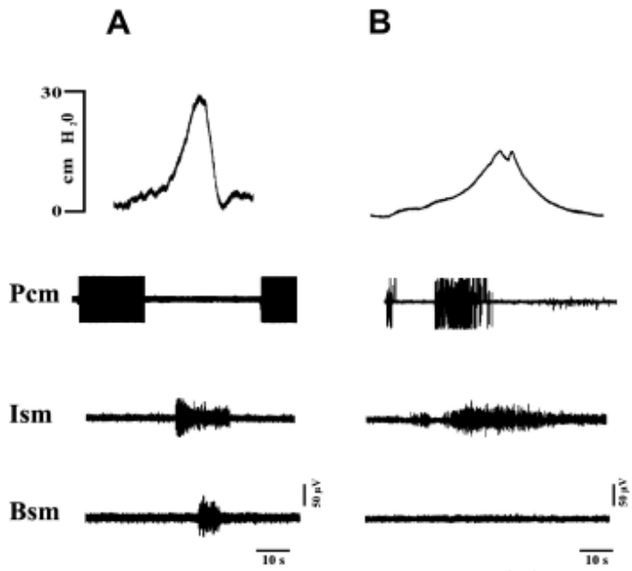

Fig. 7. Cystometrograms and EMGs of the Pcm, the Ism, and the Bsm simultaneously recorded during micturition in virgin (A) and multiparous (B) young rabbits. In virgin rabbits, temporal and coordinated activation of the Pcm, the Ism, and the Bsm occurs during the micturition phases. The cystometrogram and EMG recordings were different in multiparous rabbits and a different timing in striated-muscle activation was measured. s = seconds.

In the female rabbit, the morphology of pelvic and perineal muscles is different (Fajardo et al., 2008). The Pcm is heavier and longer than the Bsm. Though there is no difference in the number of fibers composing them, the fiber composition is different between both muscles.

By using Sudan Black stain, we have found that the Pcm is mainly composed of type I (dark) and IIa (intermediate) fibers, in contrast to that of the Bsm, which is mainly of type II (light). The morphological characteristics of those muscles are modified by multiparity. Both the Pcm and the Bsm from multiparous rabbits are lighter and longer than those of the virgin females. For each muscle, the cross-sectional fiber area of the multiparous animals was lower than that of the virgin females, without any modification about the type of fiber assessed by Sudan Black stain. Furthermore, both the Bsm and the Pcm developed lower twitch and tetanic tension force in response to electrical stimulation than the muscles of nulliparous females. In female rabbits, multiparity is associated with potentially pathological changes in the morphological and functional characteristics of these perineal and pelvic muscles, possibly as a result of stretching during parturition.

5. Conclusion

The female rabbit is an adequate model to study the physiology of pelvic and perineal muscles, and to contribute in the understanding the role of pelvic and perineal muscles in both reproductive and excretory functions. By using electromyography, our group has described the reflex activity of such muscles during the vaginal contraction and micturition. In addition, we have evaluated how the reproductive experience affects the activity of pelvic and perineal muscles, and its relationship to the urodynamics.

These findings show the importance of the participation of pelvic and perineal muscles in the reproductive and excretory processes, because in both processes the muscles are activated differentially and sequentially, not activated simultaneously as a unitary mass or functional unit. Moreover, their activity is regulated by viscerosomatic reflexes that are synchronized so that they have functions in urinary continence or fetus expulsion.

Our results in the model on the multiparity are important because they show that the uncoordinated activity of the pelvic and perineal muscles, plus other effects, such as peripheral denervation, damage to fascias, ligaments, and tissues, produced alterations in the support and dysruption in the nerve pathways of micturition. This uncoordinated activity in subsequent births can cause prolongation of the second-stage of parturition and increase the risk of urinary incontinence and pelvic organ prolapse. This knowledge will increase the accuracy of models and childbirth and may provide a better understanding about the pathologies of urinary incontinence and pelvic organ prolapse.

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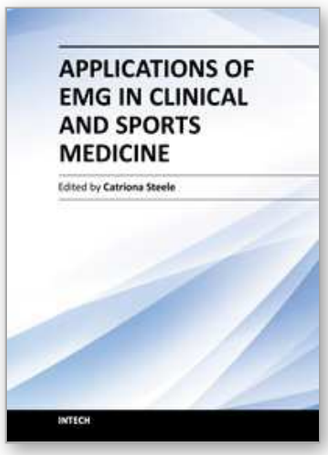

Applications of EMG in Clinical and Sports Medicine

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This second of two volumes on EMG (Electromyography) covers a wide range of clinical applications, as a complement to the methods discussed in volume 1. Topics range from gait and vibration analysis, through posture and falls prevention, to biofeedback in the treatment of neurologic swallowing impairment. The volume includes sections on back care, sports and performance medicine, gynecology/urology and orofacial function. Authors describe the procedures for their experimental studies with detailed and clear illustrations and references to the literature. The limitations of SEMG measures and methods for careful analysis are discussed. This broad compilation of articles discussing the use of EMG in both clinical and research applications demonstrates the utility of the method as a tool in a wide variety of disciplines and clinical fields.

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