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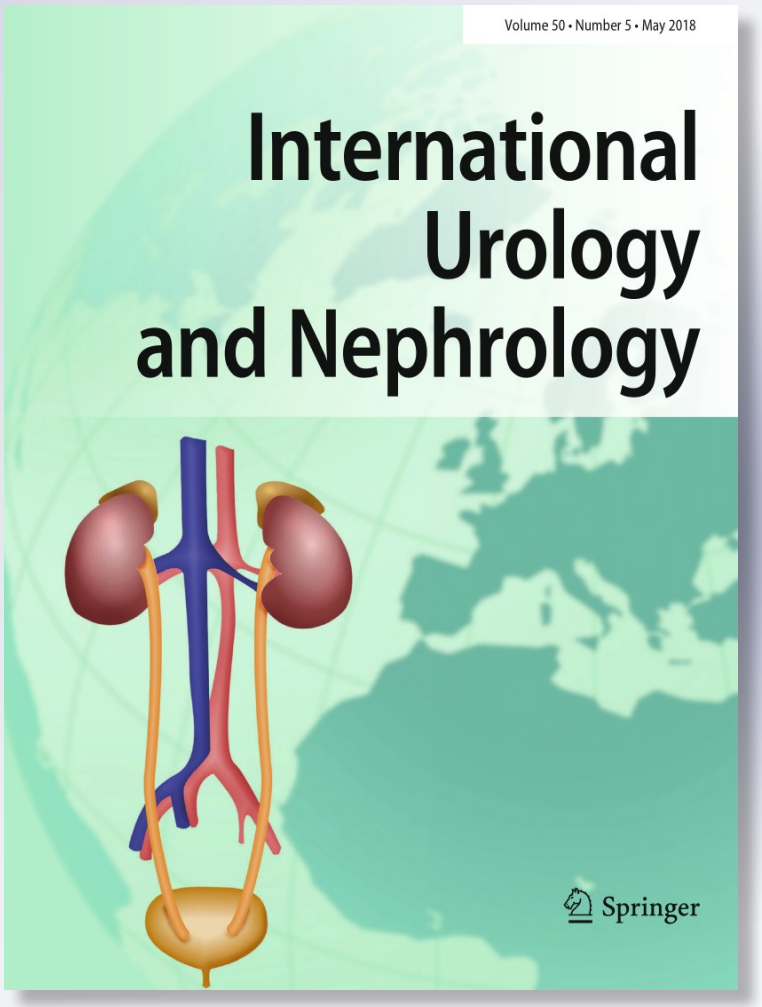

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UROLOGY - ORIGINAL PAPER

Effect of pregnancy on cooling tone and rhythmic contractions of the rat urinary bladder

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Abstract

Objectives Pregnancy is a physiological alteration that can affect urinary bladder. Cooling of urinary bladder smooth muscle is known as a potent stimulus to micturition due to an increase in muscle tone. The current study investigates the effects of pregnancy on cooling tone and on the rhythmic contractions of the urinary bladder.

Methods Twenty-four rats were used in this study as control group (non-pregnant) and pregnant group (18–20-day pregnancy). Isolated rat urinary muscle strips were suspended in organ baths containing Krebs' solution for isometric tension recording.

Results Cooling from 37 to 5 °C induced a rapid and reproducible increase in basal tone, proportional to cooling temperature. Cooling also increased the rhythmic activity (amplitude and frequency) at 30 and 25 °C, then decreased at 20 °C, and abolished at 15–5 °C. These responses were more pronounced in pregnant group than in control group. Rhythmic contractions were abolished in calcium-free, EGTA (1 mM)-containing Krebs' solution and in the presence of nifedipine, while they were not affected by CPA or TTX in both groups. Our investigation showed that the influx of extracellular calcium is important in inducing the rhythmic contractions.

Conclusions Pregnancy increases cooling-induced contraction in pregnant rat urinary preparations and its rhythmic contractions including amplitude and frequency than non-pregnant rat. Rhythmic contractions are myogenic in nature and highly extracellular calcium dependent. They may play a crucial role in urinary bladder overactivity and incontinence during pregnancy.

Keywords Pregnancy · Urinary bladder · Cooling · Rhythmic contractions · Calcium · Incontinence

Introduction

Pregnancy is characterized by profound changes in almost every organ system in order to accommodate the demands of the gestation period. The urinary tract is one of the many organ systems affected by these changes, and these may include anatomical changes, changes in hemodynamics and in circulating hormones. In pregnancy, the expanding uterus puts pressure on the bladder which experiences plastic changes [1]. Many studies evaluated the effects of hormones in the female continence mechanism have shown that the urinary tract is a target organ for the hormonal changes during pregnancy [2–12]. Urinary incontinence is a common problem and has been attributed, at least in part, to changes in bladder function and pregnancy [12]. Urinary incontinence during pregnancy can also be the result of an overactive bladder. Females who have an overactive bladder need to urinate more than usual because their bladders have uncontrollable spasms. It is known that smooth muscle of the urinary bladder exhibits spontaneous action potentials [13, 14], a phenomenon thought to underlie spontaneous rhythmic activity. Alterations in neural and rhythmic mechanisms may underline dysfunction of bladder smooth muscle contractility precipitating in bladder control disorders such as overactive bladder [15]. It was determined that the incidence of rhythmic contractions is greater in detrusor strips taken from clinically unstable bladder than in tissues taken from patients with normal bladders. This can prove that increased rhythmic contractions play a key role in bladder overactivity [16, 17]. The physiological significance of the

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spontaneous rhythmic contractions is related to the potentiality of small contractions to become an epicenter for the development of large contractions leading to urinary bladder instability. When the urinary bladder begins rhythmic contractions, which gives a sensation of urgency, the rhythmic contractions intensify. If the contractions are strong enough, they can cause micturition reflex.

Cooling has been shown to have variable effects on smooth muscle in different body organs including urinary bladder, such as airways, aorta, pulmonary artery, carotid artery and gastrointestinal tract [18–24]. Mustafa and Thulesius [23] showed cooling-induced contraction in rat urinary bladder which is inversely proportional to temperatures, and it is dependent on extracellular calcium.

The aim of the present study was to examine the effects of pregnancy on the tone of the isolated rat urinary bladder to different cooling temperatures and its effect on the rhythmic contractions.

Materials and methods

Animals

Twenty-four adult female Sprague–Dawley rats matched by date of birth and weighing approximately 200–250 g were divided into two groups: control group (eighteen non-pregnant rats) and pregnant group (six rats; 18–20-day pregnancy). Experiments were done in accordance with guidelines approved by the Institutional Animal Care and Use Committee of Kuwait University. The rats were housed on a 12-h light/dark cycle (lights on from 0600 to 1800 h). The ambient temperature was kept at 21 °C, and the rats had free access to standard laboratory food and tap water.

Preparation of bladder strips

Sodium pentobarbital (60 mg/kg; IP) was used to anaesthetize the rats followed by exsanguination, and the abdomen was opened through a midline incision to expose the bladder. The urinary bladder was removed and mounted in Krebs' solution of the following composition (mM): (NaCl 118, $MgSO_4$ 1.2, KCl 5.9, glucose 11.1, $NaHCO_3$ 26, KH_2PO_4 1.2, and $CaCl_2$ 2.2 in mM concentration) at pH 7.4. The bladder was cut longitudinally into two similar halves. The strips were 10 mm in length and 5 mm in width and then were suspended in 10-ml organ baths filled with Krebs' solution, at 37 °C and continuously gassed with a 95% O_2 and 5% CO_2 mixture. The preparations were allowed to equilibrate under optimal resting tension of 1 g for up to 60 min. The bath fluid was changed twice before starting the experiment. Isometric contractions were recorded by computerized, automated isometric transducer system (Schuler organ bath 809; Hugo Sachs Electronik, March–Hugstetten, Germany) which connected to a Gould recorder (Gould Instrument Inc., Cleveland, OH, USA), and allowed to equilibrate for 60, during which time they were washed twice. For the blockers study, we used each urinary strip preparation one time. (Four different strips from four different rats were used to have the average result to each blocker.) At the end of each experiment, the muscle was dried by filter paper and weighed, and responses were calculated as $mg \times mg^{-1}$ tissue weight. The frequency of the rhythmic contractions was calculated as the number of contractions per a minute.

Cooling protocol

The organ bath temperature was reduced using a cooling circulator bath (Haake F3, Fisons, Germany) that had been set to the appropriate temperature. It took 2–3 min to reach the desired temperature, from 37 to 30 °C then down to 5 °C, in decrements of 5 °C. Each cooling period was maintained until a peak response had leveled off before further temperature reduction. In experiments conducted with drugs, the appropriate concentration of each, which is commonly used in many previous studies, was added to the organ baths and allowed to equilibrate with the tissues for 30 min before decreasing the temperature.

Drugs

Nifedipine, cyclopiazonic acid (CPA), tetrodotoxin (TTX), ethylene glycol bis (β-aminoethylether)-*N*,*N*,*N*,*N*,-tetraacetic acid (EGTA) were purchased from Sigma chemicals, St Louis, MO, USA.

Calculations

Data are calculated as the mean of (n) experiments ± SEM, where n is the number of animals used. The differences between two mean values were analyzed using Student's t test paired or unpaired as appropriate was used. The difference was considered significant at $P < 0.05$.

Results

Cooling-induced contraction (CIC)

The urinary bladder smooth muscle strips from both control and pregnant groups maintained a steady baseline at 37 °C. Lowering the bath temperature induced rapid and reproducible increase in tone, proportional to the cooling temperature. The responses from pregnant group were significantly greater than those from control group at all used temperatures, as shown in Fig. 1a–c. Figure 1a, b

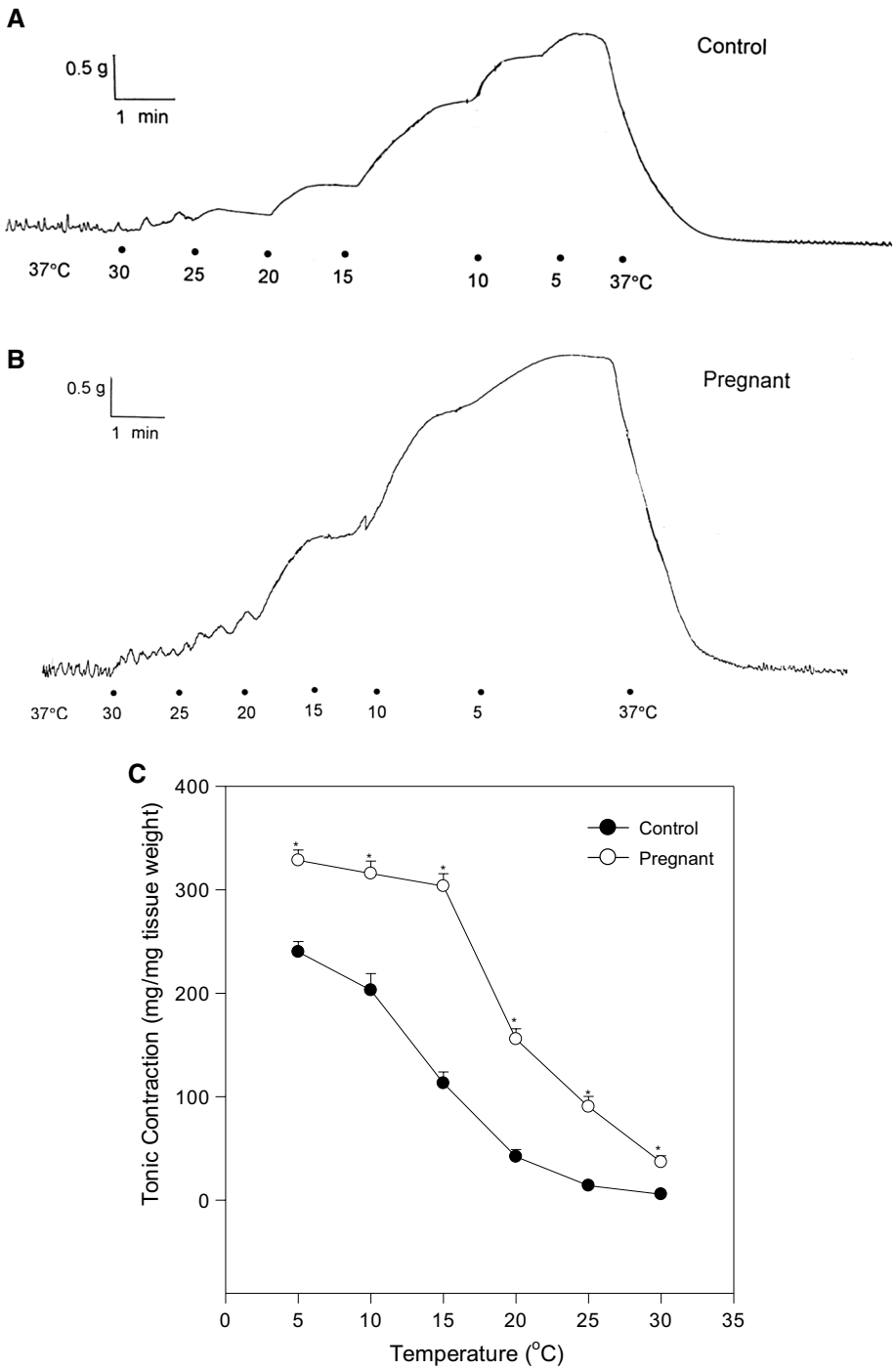

Fig. 1 **a** Original recording of cooling-induced contraction in isolated urinary bladder strips from control rat. **b** Original recording of cooling-induced contraction in isolated urinary bladder strips from pregnant rat. **c** Cooling-induced tonic contraction in an isolated rat urinary bladder strips from control and pregnant groups, showing the effect of graded cooling on force of contraction. Each point represents the mean ± SEM of 6 rats. $*P < 0.05$

shows original recordings of cooling-induced contraction in isolated urinary bladder strips from control and pregnant rats, respectively. They showed the effect of tone, amplitude and frequency of step-wise cooling in both control and pregnant rats. When the temperature was adjusted to 37 °C, the tone returned rapidly to basal levels. Highest tone was achieved at a temperature of 5 °C for the two groups.

Rhythmic contractions and cooling

All urinary bladder strips maintained consistent baseline around which there were rhythmic contractions. The rat urinary bladder smooth muscle exhibited rhythmic contractions which were regular in amplitude and frequency. They started spontaneously in bladder strips mounted in the organ bath for control and pregnant groups. Tetrodotoxin (3 μM) had

no effect on CIC and rhythmic contractions, indicating that it is myogenic in origin, as shown in Table 1. Rhythmic contractions were affected by the change in temperature. They increased at 30 and 25 °C, while they decreased at 20 °C, and were abolished at 15–5 °C. When cooled below 25 °C, the rhythmic contractions diminished in both the control and pregnant groups and disappeared after 20 °C. When the temperature was reset to 37 °C, the rhythmic contractions rapidly returned to its initial levels. The effect of cooling is significantly more on pregnant group than in control group.

Amplitude

The amplitude is the height from the steady level to the highest point of contraction. The amplitude of rhythmic contractions in both control and pregnant groups increased at 30 and 25 °C, while it decreased at 20 °C, and was abolished at 15–5 °C. The effect of cooling on the urinary strip's amplitude is significantly more in pregnant group than in control group at 37 °C and at all used temperature, as shown in Fig. 2.

Frequency

Frequency is the rate of contraction that occurs over a minute. The frequency of rhythmic contractions in both pregnant and control groups increased at 30 and 25 °C, while it decreased at 20 °C and was abolished at 15–5 °C. The effect of cooling on the urinary strips frequency is significantly more in pregnant group than in control group at 37 °C and at all used temperature, as shown in Fig. 3.

Effect of calcium

To examine the effect of calcium on the rhythmic contraction of urinary bladder strips (amplitude/frequency), we used calcium-free, EGTA (1 mM)-containing Krebs' solution, nifedipine and CPA, as shown in Table 1.

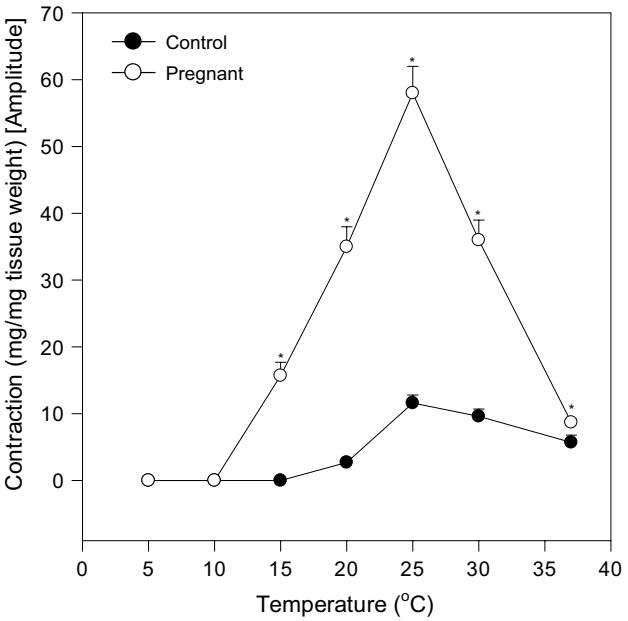

Fig. 2 Effect of graded cooling on the amplitude of rhythmic contractions in an isolated rat urinary bladder strips from control and pregnant groups. Each point represents the mean ± SEM of 6 rats. *$P < 0.05$. Notice that the amplitude of rhythmic contractions in both control and pregnant groups increased at 30 and 25 °C, while it decreased at 20 °C and was abolished at 15–5 °C. The effect of cooling on the urinary strips amplitude is significantly more in pregnant group than control group

Effect of extracellular calcium

After obtaining control responses in normal Krebs' solution, the tissues were again incubated for 30 min in calcium-free, EGTA (1 mM)-containing Krebs' solution. There was marked decrease in cooling-induced contractions. Incubation in calcium-free, EGTA (1 mM)-containing Krebs' solution abolished the amplitude and frequency of the rhythmic contractions as shown in Table 1.

Table 1 Effect of TTX and various calcium drugs on rhythmic contractions (amplitude/frequency) of rat urinary bladder at 37 °C

Condition	Amplitude mg/mg tissue weight		Frequency/min		Number of animals
	Before	After	Before	After	
Control	3.9 ± 0.7	3.8 ± 0.7	5.2 ± 0.3	5.4 ± 0.3	4
TTX (3 μM)	4.0 ± 0.9	3.8 ± 1.0	5.2 ± 0.2	5.0 ± 0.4	4
Ca^{2+}-free (1 mM EGTA)	3.9 ± 0.4	0*	5.2 ± 0.7	0*	4
Nifedipine (1 μM)	3.4 ± 0.8	0*	4.5 ± 0.2	0*	4
CPA (1 μM)	3.9 ± 0.4	4.2 ± 0.2	5.2 ± 0.7	5.2 ± 0.6	4

Before = before pretreatment with the drug

After = after 30-min incubation with the drug

TTX = tetrodotoxin, *EGTA* = ethylene glycol tetraacetic acid, *CPA* = cyclopiazonic acid

*$P < 0.05$ versus before drug

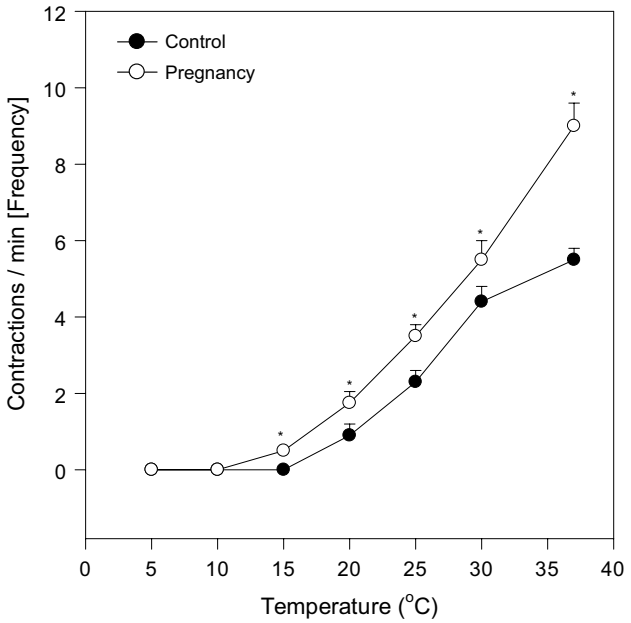

Fig. 3 Effect of graded cooling on the frequency of rhythmic contractions in an isolated rat urinary bladder strips from control and pregnant groups. Each point represents the mean ± SEM of 6 rats. *$P < 0.05$. Notice that the frequency of rhythmic contractions in both pregnant and control groups increased at 30 and 25 °C, while it decreased at 20 °C and was abolished at 15–5 °C. The effect of cooling on the urinary strips frequency is significantly more in pregnant group than control group

Effect of calcium channel blockers

The L-type calcium channel blocking agent, nifedipine (1 μM) was used. After obtaining control responses, the tissues were again incubated for 30 min in the presence of calcium channel blocker. Nifedipine abolished the rhythmic contraction in all the urinary bladder strips. The inhibition of rhythmic contractions supports the notion that contractility depends on the increase in calcium entry caused by membrane depolarization during action potentials.

Effect of intracellular calcium

CPA (10 μM), a sarcoplasmic reticulum Ca^{2+} ATPase inhibitor, has no effect on the resting basal tone and had no effect on the rhythmic contractions, amplitude and frequency.

Discussion

In the present study, we further evaluate the effects of cooling-induced contraction in the urinary bladder during pregnancy. We showed that isolated urinary bladder strips are inversely proportional to temperature, and this effect is higher in the pregnant group than in control group. The effect of cooling is more pronounced during pregnancy. Despite the importance of rhythmic contractions to urinary bladder function, little is known about it.

Spontaneous rhythmic contractions of urinary bladder smooth muscle have been demonstrated in several mammalian species and in man [25–27]. This study showed that rhythmic contraction is resistant to tetrodotoxin, suggesting that the stimulus for rhythmic contractions originates in the smooth muscle itself, so it is myogenic in nature. This result is similar to previous results done in other species [15–28]. Spontaneous rhythmic contractions have been shown to occur more commonly and to be of greater frequency and amplitude in muscle strips obtained from patients with clinically diagnosed detrusor instability and hyperreflexia compared with normal controls [16]. The present study showed that rhythmic contraction including frequency and amplitude components is also greater during pregnancy. Therefore, increased spontaneous rhythmic contractions may play a role in the pathogenesis of detrusor instability and may result in overactive bladder. Overactive bladder is characterized by urgency, frequency and incontinence. These effects are considered as a great problem during pregnancy. There is a relationship between the urinary bladder temperature and urine flow rate. Cooling can increase the urination frequency rate leading to overactive bladder. Fallis [29] indicated that heat loss of 0.05 °C in bladder temperature significantly increases urine flow rate by tenfold. Other studies showed that sudden whole body cooling can induce frequent urination [30].

Contraction of the urinary bladder smooth muscle is regulated by intracellular calcium levels and modulated by calcium sensitization pathways [26]. In our previous study, we have shown that the major source of activator calcium for cooling-induced contraction is the extracellular source, whereas calcium release from sarcoplasmic reticulum did not contribute to contractions to a major extent [23]. It is confirmed also in this study that nifedipine, organic calcium channel blocker or incubation in calcium-free, EGTA (1 mM)-containing Krebs' solution, completely abolished the rhythmic contractions, eliminated the amplitude and the frequency. Therefore, extracellular calcium is crucial to rhythmic contractions. While CPA, the sarcoplasmic reticulum Ca^{2+} ATPase inhibitor, has no effect on rhythmic contraction indicating that sequestration of intracellular Ca^{2+} did not contribute in inducing this effect. The present results suggest that in rat urinary bladder smooth muscle, the maintenance of rhythmic contractions can be attributed to increase in cellular calcium levels via voltage-dependent calcium channels.

The physiological role of spontaneous rhythmic contractions is not well studied. Our research clarified that increased spontaneous rhythmic contractions during pregnancy and its potentiation during cooling may play an important role in the pathogenesis of detrusor instability, and modulation of

these contractions may reduce or prevent the symptoms of a clinically unstable bladder during pregnancy.

In conclusion, this study has confirmed that during pregnancy the urinary bladder smooth muscle of the rat generates larger contractions in response to cooling temperatures and to rhythmic contractions. It also demonstrated that rhythmic contractions are myogenic in nature and depend on calcium influx through calcium-dependent channel. In addition, this study has interestingly shown that rhythmic contractile response can contribute in urinary bladder overactivity and incontinence during pregnancy. New drugs should act on rhythmic contractions to help in avoiding urinary incontinence during pregnancy.

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Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee of Kuwait University institution at which the studies were conducted.

Human and animals rights This article does not contain any studies with human participants performed by any of the authors.

Informed consent Informed consent was obtained from only myself because I am sole author in the study.

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