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Effects of bisphenol A and bisphenol F on porcine uterus contractility

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Abstract

Introduction: Bisphenols, as endocrine disruptors, may cause a wide range of health problems in humans, but so far, not all of them have been confirmed in animals, including pigs. Since animals are also exposed to bisphenols, we hypothesised that these substances may have an effect on uterine contractility in pigs. Therefore, the aim of the study was to investigate the effect of the most-used bisphenol, bisphenol A (BPA), and a selected analogue, bisphenol F (BPF), on the contractile activity of the pig uterus. **Material and Methods:** The investigation utilised smooth muscles from immature pigs (n = 6), cyclic pigs on days 12–14 of the oestrous cycle (n = 6) or early pregnant pigs on days 12–16 of pregnancy (n = 6). Strips of the myometrium were exposed to BPA and BPF at concentrations of 10^{-13}–10^{-1} M. Smooth muscle contractility was determined with equipment for measuring isometric contractions. **Results:** BPA caused a significant decrease in contraction amplitude, and frequency and in myometrial tension in all groups examined. BPF caused a decrease in the amplitude and frequency of contractions in all groups and in myometrial tension in the early pregnant group. **Conclusion:** The obtained results indicate that both BPA and BPF relaxed the porcine myometrium, but these changes, especially in the amplitude and frequency of contractions, were more evident after BPF treatment. The extent of relaxation is dependent on the physiological status of the animals.

Keywords: bisphenols, uterus, immature, cyclic, early pregnant gilts.

Introduction

Bisphenol A (BPA) is one of the compounds most commonly used in everyday products, *e.g.* plastics, plastic bottles, cans, food packaging, dental materials, medical equipment, thermal paper, toys and articles for children (41). Its analogue, bisphenol F (BPF), also has a wide range of applications, such as in varnishes, liners, adhesives, plastics and water pipes, as well as in dental sealants, prostheses, tissue substitutes and coatings for food packaging (6).

Both bisphenols primarily affects the human endocrine system and BPA also predisposes those exposed to the occurrence of diseases of civilisation, such as obesity and type 2 diabetes (33). Bisphenol A can cause allergies, irritate the respiratory system (32), increase blood pressure (20) and disrupt the secretion and metabolism of steroid hormones (23). It may raise the risk of breast and ovarian cancers (41) and endometriosis (17). The molecular structure of BPA is similar to the female hormone oestrogen and has a negative effect on the reproductive system, hindering conception, and predisposing women to polycystic ovary syndrome, girls to premature puberty or men to reduced sperm quality (35). Foetuses and infants are particularly vulnerable to BPA exposure. This compound can migrate from mother to foetus and affect foetal development, causing birth defects and compromising the immune system (9).

The influence of BPA on the animal reproductive tract is less documented. It has been shown that BPA increases testosterone and estradiol production and decreases progesterone levels, reduces ovarian weight and the number of corpora lutea, increases the number of atretic follicles, and reduces the number of antral follicles in the ovaries of adult female rats during

adolescence (11). Moreover, exposure to BPA resulted in a shortening of the time interval between the rise in the estradiol concentration and the pre-ovulatory luteinising hormone (LH) surge compared to the controls and generated changes in the number of follicles in the offspring of pregnant Suffolk sheep (42). It has also been shown that BPA upregulates the secretion of vascular endothelial growth factor (VEGF) in the granulosa cells of porcine ovaries, which plays a role in angiogenesis, promoting endothelial cell growth and permeability. The stimulating effect of BPA on the production of VEGF may have negative effects, leading to uncontrolled neovascularisation and, consequently, the development of pathological processes (12). Furthermore, it has been shown that high doses of BPA in adult female mice reduced the number of embryonic implantation sites, delayed the transfer of embryos from the fallopian tube to the uterus, damaged pre-implantation blastocyst development and inhibited implantation (30, 43). Moreover, alterations in the expression of three genes: homeobox A13 (HOXA13), Wnt Family Member 4 (WNT4), and Wnt Family Member 5A (WNT5A) were observed in the uteri of macaque foetuses whose mothers were exposed to BPA in the third trimester of pregnancy. Disturbed functions of the HOXA13 gene can cause hand-foot-uterus syndrome, characterised by hand and foot dysplasia and abnormalities in the female and male urogenital systems. Furthermore, disturbances in the WNT genes function may result in Müller's aplasia, depletion of the ovarian follicles and hyperandrogenism in females (7). In the offspring of mice exposed to low doses of BPA in the prenatal period, a higher frequency of uterine proliferative changes were observed, in particular, atypical uterine stromal hyperplasia, polyps and cervical sarcoma (29). Moreover, thicker uterine epithelia and stroma, and diminished epithelial apoptosis were also observed in adult offspring whose mothers were exposed to BPA during pregnancy and lactation (27). Bisphenol A has also been shown to reduce the density of tubules in the uterus (the shell gland) of adult White Leghorn chickens compared to the control group (44).

Knowledge of the influence of BPA on uterine contractility is limited but research indicated that it may reduce the amplitude and frequency of contractions in immature (2) and cyclic rats in a dose-dependent manner (13, 15). It was also demonstrated that the uteri of cyclic rats exposed to BPA showed a reduced response to acetylcholine for induction of contractions (14). Lower force of contraction was observed in cyclic rat uteri exposed to BPA and pre-contracted with prostaglandin $F_{2\alpha}$, acetylcholine (ACh) and oxytocin (36). Furthermore, BPA has been shown to reduce the amplitude and frequency of contractions of the feline uterus during the oestrous cycle (21). All of the above data indicate that BPA has a relaxative action on the myometrium.

Single reports in the literature indicate that bisphenol F (BPF) is as hormonally active as BPA and disrupts the endocrine system, demonstrating oestrogenic, androgenic and thyroidogenic effects (34). Stroheker *et al.* (40) showed that BPF increased uterine weight (both relative wet and dry weights) in immature Wistar rats. Other studies discovered that BPF added testicular mass in rats, indicating androgenic activity, made thyroid glands heavier and changed thyroid hormone concentrations (16). Recently, it was found that BPF lessened the weight of the uterus and ovaries, raised testosterone and lowered estradiol, LH and follicle-stimulating hormone levels, decreased the number of corpora lutea and antral follicles, and increased the number of atretic and pre-ovulatory follicles in adult female rats (18). To date, there are no literature data describing the effects of BPF on uterine contractile activity.

Uterine contractility is regulated by complex interactions between many factors. It also depends on the physiological and /health status of the animal. It was shown that acetylcholine chloride (ACh) raised the tension and frequency of contractions and diminished the amplitude of contractions more in the myometrium collected from early pregnant pigs (12–14 days of gestation) than in this tissue from cyclic pigs at 12–14 days into the oestrous cycle (23). In turn α-adrenergic receptors played a dominant role in triggering contractile activity induced by noradrenaline (NA), while the stimulation of β-adrenergic receptors inhibited uterine contractile activity in pigs (24, 26). It was also learned that vasoactive intestinal peptide inhibited the contractile activity of the uterus in non-pregnant and first-trimester women as well as those delivering at full term (5). Calcitonin gene-related peptide also prevented spontaneous and acetylcholine-induced uterine contractions in pregnant CD1 mice (28). In contrast, substance P stimulated uterine contractions in Sprague Dawley rats in a dose-dependent manner (39). Also neuropeptide Y, acting through receptor subtype 1, stimulated the contractile activity of the uterine muscle of Wistar rats (4). Another study reported that prostacyclin intensified the contractions of the inflamed porcine uterus on day 8 of the oestrous cycle, while it decreased them on day 16 (19).

Because data is lacking in the available literature on the effect of BPA or its analogue BPF on the contractility of the porcine uterus, an attempt was made to investigate their influence on the amplitude and frequency of myometrial contractions and on myometrial tension with data collected from immature, cyclic and early pregnant gilts.

Material and Methods

Reagents. The reagents needed for the preparation of Krebs-Ringer buffer (NaCl, KCl, $CaCl_2$, $MgCl_2$, $NaHCO_3$, NaH_2PO_4 and glucose) were purchased from Chempur (Piekary Śląskie, Poland). Acetylcholine chloride, BPA, BPF and dimethylsulfoxide (DMSO)

were purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of ACh at 10^{-4} M concentration was prepared in distilled water and the final dilutions were made in deionised water. A stock solution of BPA and BPF at 10^{-1} M concentration was prepared in DMSO and serial dilutions were made with deionised water on the day of the experiment.

Animals. The experimental groups consisted of Large White × Polish Landrace gilts divided into three groups (n = 6 in each). The first group was comprised of sexually immature gilts of 4–5 months old and weighing 60–70 kg. The second group was cyclic gilts of 7–8 months old and weighing 115–130 kg on days 12–14 of the oestrous cycle (the luteal phase). The oestrous cycle phase was confirmed on the basis of the ovarian morphology (1). The uteri were collected from animals destined for commercial slaughter and meat processing. In the third group there were pregnant gilts on days 10–16 of pregnancy (the implantation window) aged 7–9 months and weighing 120–140 kg. The procedure of selection and insemination of the gilts in this group was described previously (22). Pregnancy was confirmed by the presence and morphology of embryos in both uterine horns (3). The procedure of animal synchronisation (in the third group) and the slaughter in the local abattoir were conducted in accordance with the ethical standards of the Animal Ethics Committee of the University of Warmia and Mazury in Olsztyn.

Preparation of uterine strips and measurement of their contraction. The uterine strips used in the study were prepared as previously described (19, 25). Myometrium was obtained by shedding the endometrial layer. Strips of myometrium sized 3 × 5 mm were collected from the acquired tissues of each gilt from the middle part of the right and left uterines. The strips obtained were then washed with saline and mounted between two stainless steel hooks in Schuler organ bath (Type 809; Hugo Sachs Elektronik, March-Hugstetten, Germany) with a resting tension of 10 mN. The strips were suspended in a 5 mL water bath containing Krebs-Ringer solution at 37°C and pH 7.4 with the following composition (mmol/L): NaCl, 120.3; KCI, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 15.5 and glucose, 11.5. During the experiment, the solution was continuously saturated with a mixture of (95% O_2 and 5% CO_2). An F-30 type 372 force transducer (Hugo Sachs Elektronik) with a type 570 bridge connector was used to measure the contractions of the uterine muscle strips. Graphical recordings were made with data acquisition hardware using HSE-HA ACAD/W software (both products of Hugo Sachs Elektronik).

Schedule of contractile activity examination. The treatment scheme of uterine strips is presented in Fig. 1. The recording was started after prior equilibration for 60–90 min. At the beginning of the study, the strips were incubated with concentrations of ACh increasing from 10^{-5} to 10^{-4} M to determine the viability of the tissues and their suitability for further studies. The strips were then stimulated with concentrations of BPA or BPF increasing from 10^{-13} to 10^{-1} M administered at 15-min intervals. At the end of each measurement, the uterine strips were washed three times with 15 mL of phosphate buffer. Finally, ACh was repeatedly administered at concentrations increasing from 10^{-5} to 10^{-4} M to determine tissue viability. Only those results for which the difference in response to ACh stimulation at the beginning and at the end of treatment was less than 20% were included in the statistical analysis (24).

Pharmacodynamic analysis. The dose-response relationship between pharmacodynamic (PD) endpoints (the tension, amplitude and frequency of contractions) and bisphenol concentration was analysed by nonlinear regression with automatic outlier elimination (Q = 10%). All calculations were performed using GraphPad Prism version 8.4.2 (GraphPad Software, San Diego, CA, USA). In the first phase, exploratory dose-response analyses were conducted using various models. Based on the Akaike information criterion (AIC) and root mean square error (RMSE) value, model representing best fit to observed data was selected. Finally, a least-squares regression fit with standard Hill slope of −1.0 and log (inhibitor) *vs* response (three parameters) model was selected. Selected parameters were calculated: E_{max} – the maximal effect value, E_0 – the no effect value (baseline), Span – the dynamic range of the model based on E_{max} and E_0 distance, and $LogIC_{50}$ – the logarithm of the concentration of a bisphenol that gaves a half-maximal response. The equation $Y = E_0 + (E_{max}-E_0)/(1+10(X-logIC_{50}))$ describes the model, where X represents the logarithm of the concentration and Y represents the response.

Statistical analysis. The values of contractile activity of the uterine strips (tension – the resting/basic tension expressed in mN, frequency – the number of peaks observed and amplitude – the difference between the minimum and maximum value for a single contraction expressed in mN) before the application of biologically active substances (ACh and BPA) and after application were calculated for 15-min periods and the values before application taken as 100%. The results calculated for the 15-min periods after the administration of the individual substances in each dose are expressed as a percentage of the tension, frequency and amplitude of contractions measured during the pre-administration period. The statistical significance of the differences between the pre-treatment and post-treatment periods, as well as between the three study groups, was assessed by one-way ANOVA (GraphPad Prism 6.07; GraphPad Software) followed by Bonferroni's multiple comparison test. Three thresholds were adopted as significant differences for the statistics: *P < 0.05, **P < 0.01 and ***P < 0.001.

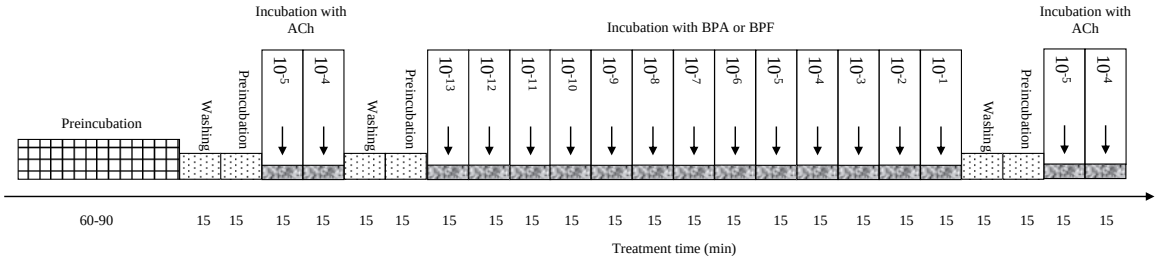

Fig. 1. Scheme presenting the sequence of treatment of the uterine strips. ACh – acetylocholine; BPA – bisphenol A; BPF – bisphenol F. Concentrations of the applied substances are given in moles

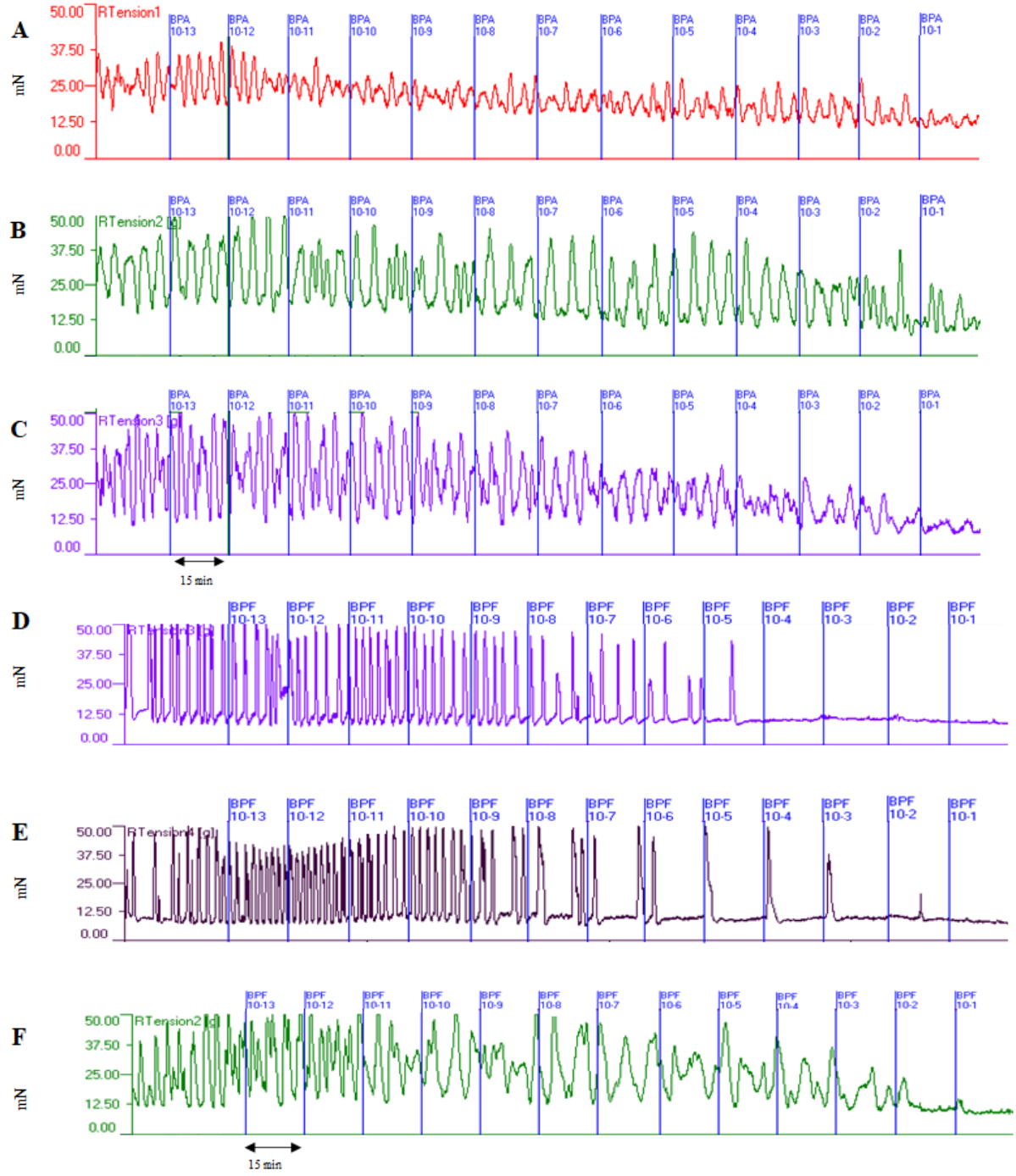

Fig. 2. Representative diagrams showing contractile activity of the myometrial strips collected from immature pigs (A and D), cyclic pigs on days 12–14 of the oestrous cycle (B and E) and early pregnant pigs on days 12–16 of pregnancy (C and F) and treated with bisphenol A (BPA) and bisphenol F (BPF) at concentrations of 10^{-13}–10^{-1} mol/L

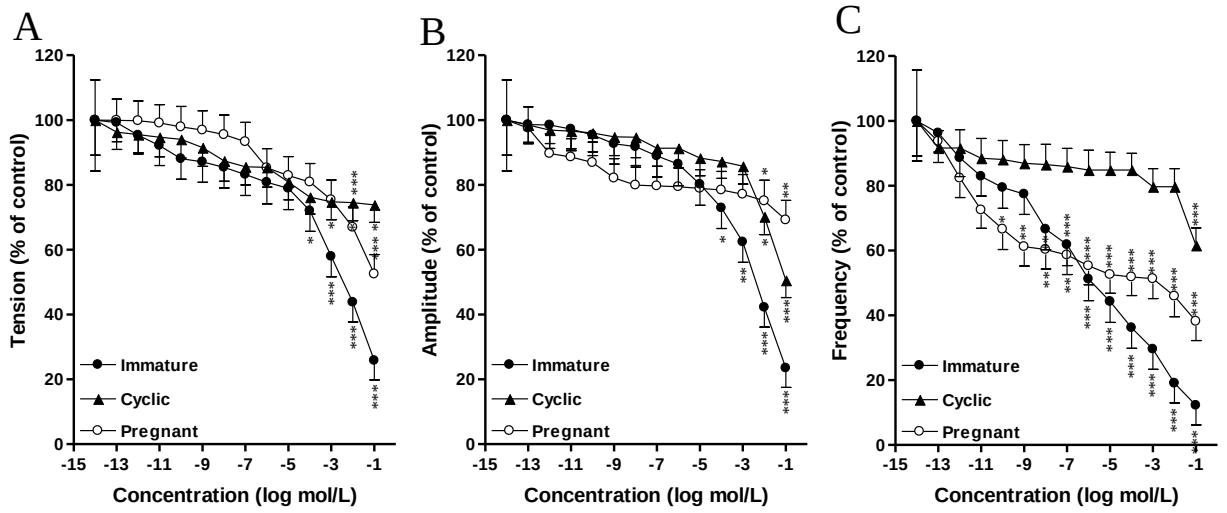

Fig. 3. Influence of bisphenol A on the tension (A), amplitude (B) and frequency (C) of contractions of the porcine uterine myometrial strips collected from immature pigs, cyclic pigs on days 12–14 of the oestrous cycle and early pregnant pigs on days 12–16 of pregnancy (n = 12 in each group). * – P < 0.05; ** – P < 0.01; *** – P < 0.001 compared to the contractile activity before the treatment

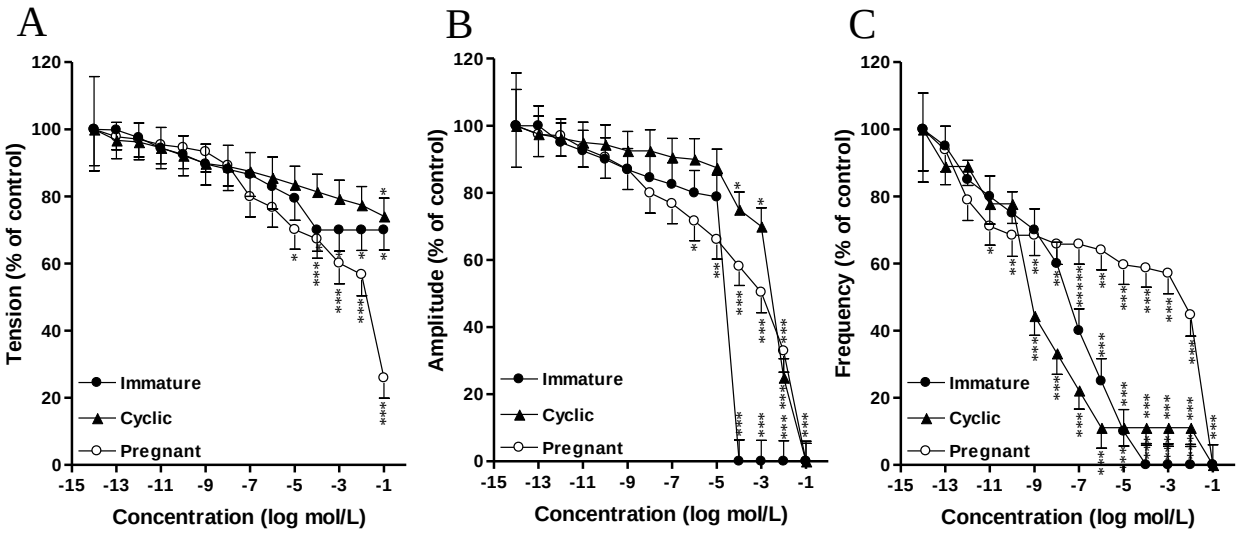

Fig. 4. Influence of bisphenol F on the tension (A), amplitude (B) and frequency (C) of contractions of the porcine uterine myometrial strips collected from immature pigs, cyclic pigs on days 12–14 of the estrous cycle and early pregnant pigs on days 12–16 of pregnancy (n = 12 in each group). *P < 0.05; **P < 0.01; ***P < 0.001 compared to the contractile activity before the treatment

Results

The contractile activity of the myometrial strips of all groups after the administration of BPA and BPF is presented in Fig. 2.

Influence of BPA on uterine contractions. Bisphenol A caused a significant decrease in tension at concentrations of 10^{-4}–10^{-1} M, 10^{-3}–10^{-1} M and 10^{-2}–10^{-1} M in the immature, cyclic and early pregnant groups compared to the pre-treatment period (Fig. 3A). The weakening in tension was significantly more pronounced in the immature group than in the early pregnant group (P < 0.05–P < 0.001) at concentrations of 10^{-3}–10^{-1} M and than in the cyclic group (P < 0.001) at concentrations of 10^{-2}–10^{-1} M. At a concentration of 10^{-1} M, the decrease in tension was significantly higher in the early pregnant group compared to the cyclic group (P < 0.001). A significant diminution in the amplitude of contractions resulted from BPA administration at concentrations of 10^{-4}–10^{-1} M in the immature group and at concentrations of 10^{-2}–10^{-1} M in the cyclic and early pregnant groups compared to amplitudes in the pre-treatment period (Fig. 3B). At concentrations of 10^{-3}–10^{-1} M, the amplitude was significantly lower in the immature group than in the cyclic group (P < 0.05–P < 0.01). The amplitude weakened significantly in the immature group compared to the early pregnant group (P < 0.001) at concentrations of 10^{-2}–10^{-1} M, and at a concentration of 10^{-1} M and compared again to the early pregnant group, the amplitude was significantly less in the cyclic group P < 0.05). Bisphenol A caused a significant slowing in the frequency of contractions

at concentrations of 10^{-8}–10^{-1} M, 10^{-1} M and 10^{-10}–10^{-1} M in the immature, cyclic and early pregnant groups, respectively, compared to the frequency in the pre-treatment period (Fig. 3C). At concentrations of 10^{-8}–10^{-1} M, the frequency of contractions was significantly lower in the immature group than in the cyclic group ($P < 0.05$–$P < 0.001$). At concentrations of 10^{-3}–10^{-1} M, the decrease in the frequency of contractions was significantly higher in the immature group compared to the early pregnant group ($P < 0.05$–$P < 0.01$). The fall in the frequency of contractions was significantly larger in the early pregnant group than in the cyclic group at concentrations of 10^{-11}–10^{-1} M ($P < 0.01$–$P < 0.001$).

Influence of BPF on uterine contractions. Bisphenol F reduced tension significantly at concentrations of 10^{-4}–10^{-1} M, 10^{-1} M and 10^{-5}–10^{-1} M in the immature, cyclic and early pregnant group, respectively, compared to tension in the pre-treatment period (Fig. 4A). At concentrations of 10^{-3}–10^{-1} M, the loss of tension was significantly higher in the early pregnant group than in the cyclic group ($P < 0.05$–$P < 0.001$). At a concentration of 10^{-1} M, tension had diminished significantly in the early pregnant group compared to the immature group ($P < 0.001$). A significant decrease in amplitude was effected by BPF at concentrations of 10^{-4}–10^{-1} M in the immature and cyclic groups and at concentrations of 10^{-6}–10^{-1} M in the early pregnant group compared to amplitudes in the pre-treatment period (Fig. 4B). At concentrations of 10^{-4}–10^{-1} M, the diminution in amplitude was significantly greater in the immature group than in the cyclic and early pregnant groups ($P < 0.05$–$P < 0.001$). At a concentration of 10^{-3} M, the suppression of amplitude was significantly stronger in the early pregnant group compared to the cyclic group ($P < 0.05$). Bisphenol F caused a significant decrease in the frequency of contractions at concentrations of 10^{-7}–10^{-1} M, 10^{-9}–10^{-1} M and 10^{-11}–10^{-1} M in the immature, cyclic and early pregnant groups, respectively, compared to frequencies in the pre-treatment period (Fig. 4C). At concentrations of 10^{-8}–10^{-2} M, the decrease in the frequency of contractions was significantly higher in the cyclic group compared to the early pregnant group ($P < 0.001$), and at concentrations of 10^{-7}–10^{-2} M, and compared again to the early pregnant group, the frequency of contractions was significantly slower in the immature group ($P < 0.001$). At concentrations of 10^{-9}–10^{-6} M, the fall in the frequency of contractions was significantly larger in the cyclic group than in the immature group ($P < 0.05$).

Pharmacodynamic analysis. The dose-response relationship between PD endpoints and the bisphenol concentration was described by a sigmoid dose-response model. The PD and model goodness-of-fit–related parameters are presented in Table 1. The presented models represented the observed data well, as indicated by the low AIC values. The root mean square error (RMSE) shows that the residuals are close to the regression line and describe the model well. The effect of BPA and BPF on the analysed PD endpoints was noted in all groups. However, the widest dynamic range of all the observed effects was in the case of amplitude changed by BPF. The highest reactivity to BPA was noted in pregnant<frequency and pregnant<amplitude. The highest reactivity to BPF was noted in relation to cyclic<tension, cyclic<frequency and immature<frequency (Table 1).

Table 1. Dose-response parameters (arithmetic mean with 90% confidence interval), based on the tension, frequency and amplitude of contractions in immature pigs, cyclic pigs on days 12–14 of the estrous cycle and early pregnant pigs on days 12–16 of pregnancy treated with concentrations of bisphenol A and F increasing from 10^{-13} to 10^{-1} M

Parameters		Tension			Amplitude			Frequency		
		Immature	Cyclic	Pregnant	Immature	Cyclic	Pregnant	Immature	Cyclic	Pregnant
E_{max}	BPA	8.887 (8.433–9.341)	6.451 (6.201–6.700)	16.73 (15.64–17.81)	24.82 (23.34–26.30)	23.09 (22.21–23.97)	27.20 (25.66–28.73)	12.74 (11.17–14.31)	6.037 (5.713–6.360)	8.235 (6.842–9.629)
	BPF	8.340 (7.948–8.731)	10.03 (9.776–10.29)	8.386 (7.657–9.114)	22.00 (21.45–22.56)	28.77 (28.23–29.31)	23.68 (21.24–26.13)	9.702 (8.885–10.52)	5.055 (4.808–5.302)	4.978 (4.687–5.270)
E_0	BPA	5.966 (5.071–6.861)	4.321 (2.913–5.728)	10.09 (7.973–12.21)	10.98 (8.672–13.28)	12.11 (10.95–13.27)	21.56 (21.01–22.11)	4.188 (3.280–5.096)	2.676 (2.122–3.229)	4.325 (3.958–4.692)
	BPF	6.756 (6.561–6.950)	8.619 (8.504–8.735)	2.383 (0.1448–4.622)	−0.07270 (−0.4302–0.2848)	−1.726 (−2.557−0.8954)	10.30 (8.076–12.53)	0.1706 (−0.2099–0.5510)	0.9818 (0.8362–1.127)	−0.7278 (−1.361−0.09438)
Span	BPA	2.921 (1.937–3.905)	2.130 (0.7245–3.536)	6.632 (4.296–8.968)	13.84 (11.24–16.45)	10.98 (9.603–12.36)	5.635 (4.026–7.245)	8.554 (6.768–10.34)	3.361 (2.752–3.970)	3.910 (2.478–5.342)
	BPF	1.584 (1.153–2.015)	1.415 (1.135–1.695)	6.002 (3.812–8.192)	22.08 (21.44–22.72)	30.50 (29.55–31.44)	13.38 (10.15–16.61)	9.531 (8.639–10.42)	4.073 (3.791–4.355)	5.706 (5.052–6.360)
$LogIC_{50}$	BPA	−3.775 (−4.633−2.916)	−1.758 (−2.769−0.7461)	−3.807 (−4.703−2.911)	−2.192 (−2.608−1.776)	−2.477 (−2.793−2.161)	−10.17 (−10.87−9.477)	−5.665 (−6.325−5.004)	−2.061 (−2.441−1.681)	−10.11 (−11.01−9.210)
	BPF	−6.622 (−7.489−5.755)	−7.314 (−7.925−6.703)	−1.634 (−2.302−0.9650)	−4.799 (−5.226−4.372)	−2.225 (−2.294−2.155)	−5.113 (−5.772−4.453)	−7.070 (−7.351−6.789)	−7.292 (−7.497−7.087)	−1.830 (−2.057−1.604)
AIC	BPA	1.554	−11.34	24.19	9.012	−5.332	−11.53	1.141	−30.13	−18.68
	BPF	−37.77	−50.61	−8.395	−19.02	−17.05	14.35	−19.84	−46.59	−32.40
RMSE	BPA	0.6702	0.4082	1.601	0.8928	0.5143	0.3188	0.6596	0.1981	0.3078
	BPF	0.1477	0.09013	0.4571	0.2268	0.3277	1.072	0.2943	0.08467	0.1816

Discussion

In the current study, the effects of various BPA and BPF concentrations on the amplitude and frequency of contractions of the porcine myometrium and its tension were investigated. To the best of the authors' knowledge, this is the first study to assess the effects of these substances on the porcine uterus. The results of the study indicated that only at high concentrations did BPA reduce the amplitude of contractions and myometrial tension in all examined groups. The frequency of contractions was already slower after the use of low concentrations of BPA in the early pregnant group but was only slower after the use of the highest concentration in the cyclic group. The obtained results indicate that the differences between the examined parameters in the three different groups are dependent on the hormonal status of the animals used in the study.

The relaxative effect of BPA indicated in the current study is in line with previous results from studies performed on other animal species. It was observed that BPA at concentrations of 10^{-6}–10^{-4} M weakened the amplitude and at the highest concentration (10^{-4} M) also diminished the frequency of the contractions of the feline uterus in the oestrous phase (21). It was also noted that BPA at a concentration of 10^{-5} M reduces the contractility of primary uterine cells in immature rats after 48 h of treatment (2). Moreover, in adult rats in the oestrous phase, and under the influence of BPA at concentrations of 10^{-7}–10^{-5} M, a dose-dependent reduction of the amplitude and frequency of spontaneous uterine contractility was observed (15). Gupta and Deshpande (14) also reported falls in the amplitude and frequency of spontaneous and acetylocholine-induced uterine contractions in rats in the oestrous phase consuming BPA 2 µg/kg/day (which corresponds to a concentration of 10^{-8} M) for 28 days compared to animals from the control group. In the current study, the amplitude of contractions was reduced by BPA at concentrations of 10^{-4}–10^{-1} M in the immature group and at concentrations of 10^{-2}–10^{-1} M in the cyclic and early pregnant groups. However, the frequency of contractions was lessened under the influence of BPA at concentrations of 10^{-11}–10^{-1} M in the early pregnant group, at concentrations of 10^{-8}–10^{-1} M in the immature group but only at a concentration of 10^{-1} M in the cyclic group. This indicates that the influence of BPA on the contractile activity of the porcine uterine smooth muscle is dependent on the physiological status of the pig, and an evident relaxative effect is generated by exposure to high concentrations of this bisphenole. The European Food Safety Authority has established a tolerable daily intake of 4 µg/kg bw/day for BPA (10), which corresponds to a concentration of 10^{-9} M. The current data and results from studies by other authors point to BPA having no relevant effect on the contractile activity of the myometrium at such a concentration.

The relaxative effect of BPA was also observed in studies using smooth muscle collected from other tissues. It was shown that BPA inhibited the contractile activity of the duodenum (37) and the distal ileum and mid colon in rats (8). This means that regardless of the origin of the smooth muscle origin it acts upon, BPA always has a relaxative effect.

BPF caused a decrease in the amplitude and frequency of spontaneous uterine contractions and in the tension of uterine muscle in the examined groups in lower concentrations than BPA, which suggests that BPF had a stronger suppressing effect on uterine contractions. There are no data on the effect of BPF on the contractility of the uterus or other organs in pigs or other species of animals in the available literature. Therefore, it is difficult to relate the obtained results to other experiments.

The observed data and final pharmacodynamic analysis in the current study represent a model with a low dynamic range (Span). Detailed model analyses were performed since, in such a case, a "visual inspection" of the collected data cannot be a foundation for conclusions. The pharmacodynamic model analysis confirmed that the differences between the pregnant, cyclic, and immature groups might not be clinically meaningful because of the relatively high variability of the observations made. In the current study, this variability was confirmed by the relatively wide ranges of confidence intervals for the IC_{50}. High variability or low dynamic range is one of the many features of the model. In the presented study, the specificity of the data made it possible to adjust the pharmacodynamic model with a high degree of fit to the observed data, which was confirmed by the low AIC value. The purpose of the model analysis was to determine the $\log IC_{50}$ value. This parameter was the lowest for amplitude in the pregnant group's samples treated with BPA and for tension in the cyclic group treated with BPF.

Although the present studies were not conducted to elucidate the mechanism by which BPA and BPF affected porcine uterine contractility, it is possible to theorise about the bisphenol mode of action from data from other studies. It was postulated that the activation of the nitric oxide pathway may be involved in the relaxation of uterine tissue (15). The results from another study suggest that norepinephrine (NE)-secreting motor neurons are involved in BPA-induced inhibition of duodenal contractions, and NE likely causes relaxation through α-adrenergic receptors located in the duodenal smooth muscle cell membrane (38). It was also demonstrated in a collagen gel uterine contraction study *in vitro* that exposure to BPA can inhibit the expression of uterine contractile proteins involving the oxytocin- and prostaglandin-related pathways (2). Moreover, it is believed that BPA may reduce the excitability of uterine muscle tissue nerve plexuses. This is confirmed by data showing that BPA lowered the action potential of the fibres of the frog sciatic nerve (31).

The normal contractile action of the non-pregnant uterus is important for semen transport in the

reproductive tract and the movement and positioning of embryos in the uterine cavity. The results of the current study establish that both BPA and BPF in high concentrations can cause problems in early pregnancy due to excessive relaxation of the myometrium.

In summary, BPA and BPF at high concentrations significantly reduced the contractile activity of porcine uterine smooth muscle, BPF having a stronger relaxative effect than BPA.

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