Polish Journal of Veterinary Sciences Vol. 13, No. 4 (2010), 597-603

DOI 10.2478/v10181-010-0009-0

Original article

Contractile effect of $PGF_2\alpha$ and PGE_2 on isolated branches of uterine and ovarian artery in different days of estrous cycle and early pregnancy in pigs

J. Skipor, M. Pikulińska, S. Stefańczyk-Krzymowska

Department of Reproductive Endocrinology and Pathophysiology,
Institute of Animal Reproduction and Food Research of Polish Academy of Sciences,
Tuwima 10, 10-747 Olsztyn, Poland

Abstract

The contractile effects of $PGF_2\alpha$ (3×10^{-6} to 10^{-4} M) and PGE_2 (10^{-7} to 10^{-5} M) were examined on isolated branches of ovarian artery (OA) and extramyometrial branches of uterine artery (UA) collected from pigs in the luteal (day 10-12) and follicular phase (day 17-20) of the estrous cycle, and during early pregnancy (day 10-12). Strong contraction was demonstrated in both arteries during all investigated periods in response to $PGF_2\alpha$, which was significantly higher ($P < 0.01$) than to PGE_2, being negligible in the follicular phase. In UA, the effective dose of $PGF_2\alpha$ (ED_{50}) amounted 7.9×10^{-6} M and 6.3×10^{-6} M in the luteal and follicular phase, and 5.0×10^{-6} M in early pregnancy. ED_{50} for PGE_2 reached 5.0×10^{-7} M in the luteal phase, and 4.1×10^{-7} M in early pregnancy. For both prostaglandins, the contraction was much stronger ($P < 0.01$) in OA than in UA branches. In OA, the ED_{50} for $PGF_2\alpha$ was 1.2×10^{-5} M in the luteal phase and was significantly higher ($P < 0.05$) than in the follicular phase (3.1×10^{-6} M) and early pregnancy (2.7×10^{-6} M). ED_{50} for PGE_2 amounted 7.3×10^{-7} M in the luteal phase and 1.7×10^{-7} M in early pregnancy. Studies showed the influence of the estrous cycle and early pregnancy on OA branches sensitivity to the contractile effect of $PGF_2\alpha$ and the lack of this effect on UA branches, and the influence of the estrous cycle on UA and OA branch contraction in response to PGE_2.

Key words: uterine artery, ovarian artery, $PGF_2\alpha$, PGE_2, pregnancy, pigs

Introduction

The uterus of cycling and pregnant pigs as well as developing embryos are the source of prostaglandin E_2 (PGE_2) and $F_2\alpha$ ($PGF_2\alpha$) that leave the uterus with lymph and venous effluent. In pigs, $PGF_2\alpha$ permeates in broad ligament vasculature from venous blood and lymph to the arterial blood and then it reaches the ovary via local destination transfer (Kotwica 1980, Stefańczyk-Krzymowska et al. 1990). Independently of this, $PGF_2\alpha$ is retrograde transferred to the uterine tissue and uterine cavity during the time of cyclic corpus luteum functioning and in early pregnancy (Krzymowski et al. 1986, 1987,

Correspondence to: J. Skipor, e-mail: j.skipor@pan.olsztyn.pl

Stefańczyk-Krzymowska et al. 1990). Our recent studies demonstrated the retrograde transfer of PGE_2 from the uterine lymph and venous blood to arterial blood supplying the uterine horn and its local destination transfer to arterial blood supplying the ovary in early pregnant (Stefańczyk-Krzymowska et al. 2005, 2006) as well as in cycling gilts (Chłopek et al. 2008).

The veno-arterial communication is a functional mechanism in the uterine circulation, which allows uterine and placental products in the venous effluent to influence directly adjacent arteries without first traversing the entire systemic circulation. A similar effect occurs in lymphatic-arterial communications, as revealed in the ewe (Heap et al. 1985, 1989) and sow (Krzymowski et al. 1986, Stefańczyk-Krzymowska et al. 2005, 2006, Chłopek et al. 2008). Celia and Osol (2002) demonstrated that phenylephrine and acetylcholine infused into isolated rat uterine vein are able to alter the tone of adjacent uterine arteries in a concentration-dependent manner. Both PGE_2 and $PGF_2\alpha$ are known vasoactive factors in the reproductive organs. It may be expected that PGs transferred to arterial blood supplying the uterus and ovary regulate the tone of small branches of uterine and ovarian arteries and therefore influence the condition of local vascular transfer of PGs as well as other substances.

Both uterine and ovarian blood supply change dramatically throughout the estrous cycle and the involvement of ovarian steroid hormones in the regulation of reproductive organ vasculature reactivity has been demonstrated (Ford et al. 1982, White 2002). However, the correlation of ovarian arterial blood flow and uterine arterial blood flow with the level of progesterone secretion during porcine estrous cycle was different (Ford et al. 1982). The presence of porcine embryos also influenced the uterine and ovarian blood flow (Ford et al. 1982) and their adaptation to the pregnancy (Chang and Zhang 2008). The genomic and non-genomic effects of progesterone and 17β-estradiol on the vascular tone of the uterine artery during the estrous cycle have been investigated in detail with respect to both classic and membrane receptors (White 2002, Brenner and Slayden 2004, Chang and Zhang 2008). It might be presumed that changes in ovarian endocrine function and embryonic secretion of steroids may affect the reactivity of uterine and ovarian vessels to prostaglandins.

Proper evaluation of PGs effects on uterine artery function under *in vivo* conditions or on an isolated whole uterus is difficult. PGs influence uterine smooth muscle tone (Maigaard et al. 1985) and cause the mechanical compression of the uterine vasculatures that change blood flow in supplying arteries. Only studies conducted on isolated blood vessel made it possible to define precisely the action of PGs on their tension.

To our knowledge no information is available on the action of $PGF_2\alpha$ and PGE_2 on small branches of uterine and ovarian arteries in gilts. Therefore, the influence of $PGF_2\alpha$ and PGE_2 on the tension of isolated 3rd order branches of uterine artery and branches of the ovarian artery of cycling and early pregnant gilts was examined in the present study and the effect of the stage of the reproductive processes on contractile reactivity of these vessels to both prostaglandins was analyzed.

Materials and Methods

Animals

All the experiments were conducted in accordance with protocol No 2/2007 approved by the local Ethical Commission for Animal Experiments. Crossbred, sexually matured gilts (n = 15, mean body weight ca100 kg), bred on a commercial farm were randomly assigned to groups of five. One group of gilts was mated after two controlled estrous cycles. Twelve days after mating, and on days 12 or 18 of the estrous cycle the gilts were killed by electrical stunning (ENZ 300, Metalowiec, Bydgoszcz, Poland) and exanguination in a local slaughter house.

Vessel preparation

The reproductive tract (uterus, ovaries and broad ligament) was excised immediately after slaughter, placed in ice cold saline and quickly transported to the laboratory. The day of the luteal and follicular phase were confirmed by morphological appearance of the ovaries and the pregnancy was confirmed by the presence of embryos. Third-order branches (OD, 150 μm) of the uterine artery supplying the oviduct and paraoviductal part of the uterine horn, and branches of the ovarian artery from the periovarian plexus were isolated. They were cleaned of loose connective tissue, cut into rings (length 3 mm) free of side branches, and placed in a Petri dish filled with ice cold saline.

Set-up and experimental protocol

Arteries were prepared for isometric tension recording according to the method described previously (Skipor et al. 2007). Randomly chosen arterial rings (n = 6, only uterine or only ovarian artery) were used for the first part of the experiment, while the remaining rings stayed in the Petri dish for a sec-

ond part of the experiment, which was conducted 5 hours later (this delay did not influence the vessel reactivity). Under stereoscopic microscope (Stemi DV4 SPOT, Zeiss, Germany) two fine (80 μm), stainless steel triangles were placed through the lumen of the vascular ring. The arteries were then fixed in 6 ml organ bath at 37°C, containing a modified Krebs-Henseleit solution (mM: NaCl, 115; KCl, 4.6; KH_2PO_4 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11). All chemicals used were obtained from Sigma-Aldrich GmbH, Germany. The pH was maintained at 7.4 by constant bubbling with 95% O_2 – 5% CO_2. One triangle was fixed to a stationary rod, while the other was hooked to a strain gauge for isometric tension recording, thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included: force transducer (F30) with vernier control (type 850 N), transducer amplifier (TAM-A), Data Acquisition Hardware with ACAD Software (all from HUGO Sachs Elektronics, Germany). After 60 min of resting, the vascular rings were stretched incrementally to their optimal passive tone of 17.5 mN, as determined previously by their contractile response to 60 mM KCl. After an equilibration period of 60 min, one part of the vessels was treated with $PGF_{2\alpha}$ (Sigma-Aldrich) and a second part with PGE_2 (Sigma-Aldrich), both given to each organ bath in a cumulative concentration manner ranging between 10^{-9} M to 10^{-4} M and 10^{-9} M to 10^{-5} M, respectively. After the highest dose of PGs, the reactivity of the vessel was tested using 60 μl of phenylephrine (Sigma-Aldrich, 10^{-4} M) corresponding with ED_{50} (10^{-6} M). Only the data of vessels reacting to phenylephrine were included in the results.

Data analysis

Dose-response curves. Data on the curves are presented as means ± standard error of the mean (SEM). For the generation of final dose-response curves, a concentration of prostaglandins was transformed to logarithms (GraphPad PRISM, USA). The final curves were transformed using the Hill equation for a sigmoid curve to give effective concentrations for a definite response (e.g. EC_{50}: a concentration producing 50% of the maximum response). Data for ED_{50} are presented as means with a 95-percent confidence interval (95%CI) for EC_{50}.

Statistics. An evaluation of the estrous cycle and pregnancy effect on the sensitivity of vessels to PGs is based on the corresponding dose-effect curves (see Dose-response curves). Sensitivities were regarded as significantly different ($P < 0.05$) if the EC_{50} values for these curves proved to belong to different entireties by the F test (GraphPad PRISM, San Diego, USA). The effect of vessel localization on the level of contraction was examined by one-way ANOVA followed by post-hoc Tukey's test (Graph Pad Prism).

Results

Cumulative doses of $PGF_{2\alpha}$ (10^{-9} M to 10^{-4} M) generated an increase in the vascular tension in third-order branches of the porcine uterine artery collected during the follicular and luteal phase of the estrous cycle and in early pregnancy (Fig. 1, left). Values of the isomertic tension of the isolated vessels depended on a dose of $PGF_{2\alpha}$ and it averaged maximally in the luteal phase, follicular phase and early pregnancy 31.7 ± 12.2 mN, 31.6 ± 8.8 mN and 26.2 ± 7.1 mN, respectively. The effective dose of $PGF_{2\alpha}$ (ED_{50}) generating a constriction of third-order branches of the uterine artery at a level of 50 per cent in the luteal phase, follicular phase and early pregnancy averaged 7.9×10^{-6} M, 6.3×10^{-6} M and 5.0×10^{-6} M, respectively. Analysis of the data with the F test demonstrated lack of influence of the phase of estrous cycle and early pregnancy on the slope of the curve dependent on the dose of $PGF_{2\alpha}$ and/or their ED_{50}. The values of ED_{50} for $PGF_{2\alpha}$ are presented in table 1.

In response to cumulative doses of PGE_2 (10^{-9} M to 10^{-5} M) the tension of third-order branches of the porcine uterine artery increased, but it was lower ($P < 0.01$) than in response to $PGF_{2\alpha}$ (Fig. 1, right). The increase in the tension of arterial branches collected in the follicular phase of the estrous cycle treated with cumulative doses of PGE_2 was small (maximally 0.9 mN) or not present. The value of isometric tension of the isolated vessels in the luteal phase and early pregnancy depended on the dose of PGE_2 and it amounted maximally to 8.8 ± 3.6 mN in the estrous cycle and 4.4 ± 3.1 mN in pregnancy. The ED_{50} of PGE_2 was established at the level of 5.0×10^{-7} M in the luteal phase of the estrous cycle and 4.1×10^{-7} M in early pregnancy. Due to negligible reactivity of arteries collected during the follicular phase to cumulative doses of PGE_2, ED_{50} was not able to be determined. Analysis of the data with the F test demonstrated the influence of the phase of the estrous cycle and a lack of influence of early pregnancy on the slope of the curve dependent on the dose of PGE_2 and/or their ED_{50}. The values of ED_{50} for PGE_2 are presented in Table 1.

In general, arterial branches from the periovarian vascular complex reacted to both prostaglandins, developing a greater ($P < 0.01$) force than that of the uterine artery branches in all studied stages of reproductive processes (Fig. 2). The maximal contractile response of isolated ovarian artery branches from the luteal phase, follicular phase and early pregnancy to

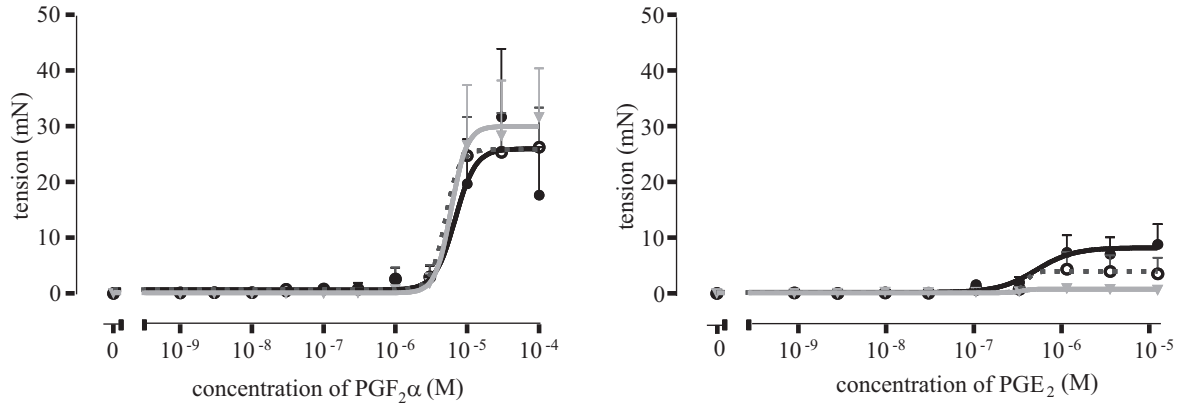

Fig. 1. Dose-response curves for $PGF_2\alpha$ (left) and PGE_2 (right) in third order branches of uterine artery from the luteal phase (solid black line), follicular phase (solid grey line) and early pregnancy (dotted black line). Error bars indicate values of SEM.

Table 1. Mean effective dose ($ED_{50} \pm 95\%$ CI) of $PGF_2\alpha$ and PGE_2 determined on the branches of uterine artery of pigs in the luteal (days 10-12) and follicular (days 17-20) phase of estrous cycle and early pregnancy (days 10-12).

Phase	$PGF_2\alpha$ ED_{50}	± 95% CI	PGE_2 ED_{50}	± 95% CI
Luteal	7.9×10^{-6} M[a]	$5.4\text{-}11.8 \times 10^{-6}$	5.0×10^{-7} M[a]	$2.1\text{-}12.0 \times 10^{-7}$
Follicular	6.3×10^{-6} M[a]	$3.4\text{-}11.8 \times 10^{-6}$	ND	ND
Pregnancy	5.0×10^{-6} M[a]	$2.7\text{-}9.3 \times 10^{-6}$	4.1×10^{-7} M[a]	$0.17\text{-}98.0 \times 10^{-7}$

ND – not detected

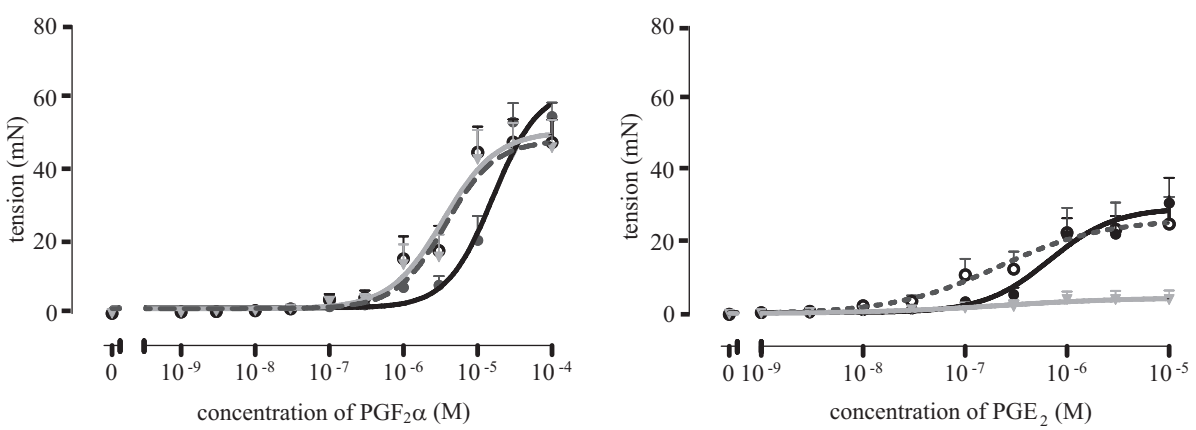

Fig. 2. Dose-response curves for $PGF_2\alpha$ (left) and PGE_2 (right) in branches of ovarian artery from the luteal phase (solid black line), follicular phase (solid grey line) and early pregnancy (dotted black line). Error bars indicate values of SEM.

Table 2. Mean effective dose ($ED_{50} \pm 95\%$ CI) of $PGF_2\alpha$ and PGE_2 determined on the branches of ovarian artery of pigs in the luteal (days 10-12) and follicular (days 17-20) phase of estrous cycle and early pregnancy (days 10-12).

Phase	$PGF_2\alpha$ ED_{50}	± 95% CI	PGE_2 ED_{50}	± 95% CI
Luteal	1.2×10^{-5}M[a]	$0.95\text{-}1.6 \times 10^{-5}$	7.3×10^{-7}M[a]	$4.4\text{-}12.0 \times 10^{-7}$
Follicular	3.1×10^{-6} M[b]	$1.9\text{-}4.9 \times 10^{-6}$	ND	ND
Pregnancy	2.7×10^{-6}M[b]	$1.8\text{-}4.2 \times 10^{-6}$	1.7×10^{-7}M[a]	$0.68\text{-}4.6 \times 10^{-7}$

ND – not detected a,b – $p < 0.05$

$PGF_2\alpha$ averaged 55 ± 3.8 mN, 46.2 ± 7.6 mN and 47.7 ± 6.3 mN, respectively. The contractile response to PGE_2 was, in all studied stages of reproductive processes, weaker than that to $PGF_2\alpha$ and it averaged in the early pregnancy and luteal phase 25 ± 7.5 mN and 31 ± 7 mN, respectively, and in the follicular phase only 4.1 ± 2.4 mN. The effective dose of $PGF_2\alpha$ in the luteal phase of the estrous cycle averaged 1.2×10^{-5} M and it was significantly higher ($P < 0.05$) than those in the early pregnancy and the follicular phase, amounting to 2.7×10^{-6} M and 3.1×10^{-6} M, respectively. The effective dose of PGE_2 established for the arteries of the periovarian vascular complex collected in early pregnancy amounted to 1.7×10^{-7} M and 7.3×10^{-7} M in the luteal phase. Analysis of the data with the F test demonstrated the influence of the phase of the estrous cycle and early pregnancy on the slope of the curve dependent on the dose of $PGF_2\alpha$ and/or their ED_{50} and the influence of the estrous cycle on the slope of the curve dependent on the dose of PGE_2. The values of ED_{50} for $PGF_2\alpha$ and PGE_2 are presented in Table 2.

Discussion

The result of the present study demonstrated the sensitivity of the branches of the porcine uterine artery and ovarian artery to uterine PGs. Both, PGE_2 and $PGF_2\alpha$ generated an increase in the vascular tension of the isolated rings of these vessels in a dose-dependent manner. Strong contractile reaction was demonstrated during the luteal and follicular phase of the estrous cycle and in early pregnancy in response to $PGF_2\alpha$, that was significantly higher ($P < 0.01$) than in response to PGE_2. Our results are in agreement with those by Maigaard et al. (1985a) indicating that in human extramyometrial arteries $PGF_2\alpha$ most potently, but also PGE_2, caused concentration-dependent contraction. Interestingly, the authors observed this effect in extramyometrial arteries, while in intramyometrial arteries the contractant effect of both PGs was negligible. According to Maigaard et al. (1985a) the reactivity of branches of the uterine artery changed with the vessel diameter and localization in relation to the miometrium. They demonstrated that uterine artery branches collected from the area adjacent to the uterine horn react to $PGF_2\alpha$, developing a tension that constituted 135% of that after KCl, while intramyometrial arteries developed tension of a level of only 6% of that developed in reaction to KCl. In our study these parameters were of a level of 88%, 92% and 83% for branches of the uterine artery and 96%, 110% and 94% for branches of the ovarian artery in the luteal and follicular phase of the estrous cycle, and early pregnancy, respectively.

For both PGs, the contraction was much stronger ($P < 0.01$) in ovarian artery branches than in uterine artery branches. This might result from better development of the muscular layer of these vessels in comparison to the uterine artery branches. We did not observe any significant differences between the maximal tension developed in response to $PGF_2\alpha$ by arteries collected during the luteal and follicular phase of the estrous cycle and early pregnancy, but we found a significant ($P < 0.01$) difference in their response to PGE_2. Arteries collected during the follicular phase of the estrous cycle react to PGE_2, developing very little tension or not reacting. The weak reactivity of uterine and ovarian artery branches to PGE_2 in the follicular phase as well as significant differences in ED_{50} for $PGF_2\alpha$ in the ovarian artery may result from the influence of the stage of the estrous cycle on the expression of contractile PGs receptors in the uterine and ovarian artery. It has been demonstrated that the effect of PGs on the tone of the smooth muscular tissues of the uterus, enabling the achievement their various physiological functions, depended on the distribution of specific prostanoid receptors: FP, EP_1, EP_2, EP_3, DP, IFP and TP (Cao et al. 2002). These authors demonstrated a contractile response of uterine tissue to $PGF_2\alpha$ and PGE_2 and the localization of contractile receptors (FP, EP_1 and EP_3) in the nonpregnant porcine uterus. The distribution of these receptors differed between uterine region and the level of mRNA and protein of the FP receptor was consistent with the contractile response (Cao et al. 2005). PGE_2 has a different affinity to EP receptors (EP_3 and $EP_4 > EP_1$ and EP_2) as was demonstrated by Kiriyama et al. (1997). Unfortunately, there is no data demonstrating the distribution of PGs receptors in uterine and ovarian arteries in pigs or the effect of the estrous cycle and pregnancy on their expression in gilts. Studies conducted on arteries collected from the uterine cervix of ovariectomized ewes demonstrated that estradiol replacement decreased EP_1 and EP_3 receptor protein in blood vessels while progesterone replacement had no significant effect on EP receptor protein expression (Schmitz et al. 2006). Our studies were conducted on vessels collected from animals in the luteal and follicular phase of the estrous cycle and early pregnancy (day 10-12 after fertilization). In this stage of pregnancy spherical 5-7 mm blastocysts are capable of enhanced synthesis of estradiol-17β and other estrogens (Fisher et al. 1985, Pusateri et al. 1990). In our study any cycle and pregnancy effect on the tension developed by both arteries in response to KCl was not observed, in contrast to the influence of the reproductive processes on the vessels sensitivity to PGs (data not presented).

In pigs, the concentration of prostaglandins (PGs) in uterine venous blood increases considerably from day 13 up to day 17 of the estrous cycle, but in this period the level of PGE_2 remains three times lower that of $PGF_2\alpha$ (Christenson et al. 1994). In pregnant gilts the concentration of PGs in uterine venous effluent increases markedly on days 11-12 after mating, with PGE_2 predominant (Christenson et al. 1994). The concentration of $PGF_2\alpha$ and PGE_2 in the uterine and broad ligament tissues changes in different stages of the reproductive processes. In cycling gilts both PGs reached a several-fold higher concentration during the follicular phase than in the luteal phase of the porcine estrous cycle (Stefańczyk-Krzymowska et al. 1994a, Chłopek et al. 2008). In early pregnant gilts the concentration of PGE_2 was several times higher, but $PGF_2\alpha$ was lower than in cycling gilts (Guthrie and Lewis 1986, Stefańczyk-Krzymowska et al 2005). In the pig, the permeation of $PGF_2\alpha$ from venous blood and lymph flowing out of the uterus to the arterial blood and its retrograde transfer to the uterine tissue is much more effective during the time of corpus luteum functioning i.e. during the luteal phase of the estrous cycle and early pregnancy than during luteolysis (Krzymowski et al. 1986, Stefańczyk-Krzymowska et al. 1990). The local destination transfer of this PG to the ovary was, in early pregnancy, significantly reduced in comparison to the luteolysis period (Stefańczyk-Krzymowska et al. 1990). However, transfer of PGE_2 to the ovary was intensified during the luteal phase of the estrous cycle and in early pregnancy and reduced in the luteolysis period (Stefańczyk-Krzymowska et al. 2005, Chłopek et al. 2008). This different distribution of PGs in reproductive organs may in turn change the function of blood vessels.

The present study demonstrated that uterine PGs affected the function of the arterial vessels of the entire broad ligament and therefore may influence uterine and ovarian blood flow. The vascular tension of these vessels is also modulated by steroid hormones, oxytocin, cytokines and neurotransmitters. The factors affecting the tension of the blood vessels changes the condition of blood circulation in the mesovarium and mesometrium, blood vessel permeability and the creation and flow of the lymph. Thus, they may influence processes conditioning local delivery of ovarian hormones to the uterus (Stefańczyk-Krzymowska et al. 1994) and uterine PGs to the ovary (Heap et al 1985, 1989, Stefańczyk-Krzymowska et al. 2006, Chłopek et al. 2008). It has been earlier demonstrated that blocking of α1-adrenoreceptors affected the local destination transfer of steroid hormones to the uterus (Stefańczyk-Krzymowska et al. 1997). Similarly, the changes in blood circulation in different stages of the reproductive processes affected the efficiency of the retrograde transfer of ovarian hormones (Stefańczyk-Krzymowska et al. 2002, Wąsowska and Stefańczyk-Krzymowska 2006) and uterine PGs (Krzymowski et al. 1986, 1987, Stefańczyk-Krzymowska et al. 1990, 2005, Chłopek et al. 2008). In summary, we demonstrated a vasoconstrictor effect of $PGF_2\alpha$ and PGE_2 on branches of uterine and ovarian arteries of pigs. In the case of PGE_2 this action was, in both arteries, affected by the estrous cycle, while the effect of $PGF_2\alpha$ on the ovarian artery was affected additionally by pregnancy.

Acknowledgement

The study was supported by the Ministry of Science and Higher Education in 2005.

References

Brenner RM, Slayden OD (**2004**) Steroid receptors in blood vessels of the rhesus macaque endometrium: a review. Arch Histol Cytol 67: 411-416.

Cao J, Shayibuzhati M, Tajima T, Kitazawa T, Taneike T (**2002**) In vitro pharmacological characterization of the prostanoid receptor population in the non-pregnant porcine myometrium. Eur J Pharmacol 442: 115-123.

Cao J, Yosida M, Kitazawa T, Taneike T (**2005**) Uterine region-dependent differences in responsiveness to prostaglandins in the non-pregnant porcine myometrium. Prostaglandins Other Lipid Mediat 75: 105-122.

Celia G, Osol G (**2002**) Venoarterial communication as a mechanism for localized signaling in the rat uterine circulation. Am J Obstet Gynecol 187: 1653-1659.

Chang K, Lubo Zhang (**2008**) Steroid hormones and uterine vascular adaptation to pregnancy. Reprod Sci 15: 336-348.

Chłopek J, Radomski M, Stefańczyk-Krzymowska S (**2008**) Retrograde and local destination transfer of uterine prostaglandin E_2 during the porcine oestrous cycle. Med Weter 64: 588-591.

Christenson LK, Farley DB, Andreson LH, Ford SP (**1994**) Luteal maintenance during early pregnancy in the pig: role for prostaglandin E_2. Prostaglandins 47: 61-75.

Fischer HE, Bazer FW, Fields MJ (**1985**) Steroid metabolism by endometrial and conceptus tissues during early pregnancy and pseudopregnancy in gilts. J Reprod Fertil 75: 69-78.

Ford SP, Reynolds LP, Magness RR (**1982**) Blood flow to the uterine and ovarian vascular beds of gilts during the estrous cycle and early pregnancy. Biol Reprod 27: 878-885.

Guthrie HD, Lewis GS (**1986**) Production of prostaglandin F_2 alpha and estrogen by embryonal membranes and endometrium and metabolism of prostaglandin F_2 alpha by embryonal membranes, endometrium and lung from gilts. Dom Anim Endocrinol 3: 185-198.

Heap RB, Fleet IR, Hamon M (**1985**) Prostaglandin $F_2\alpha$ is transferred from the uterus to the ovary in the sheep by

lymphatic and blood vascular pathways. J Reprod Fertil 74: 645-656.

Heap RB, Fleet IR, Davis AJ, Goode JA, Hamon MH, Walters DE, Flint AP (**1989**) Neurotransmitters and lymphatic-vascular transfer of prostaglandin $F_2\alpha$ stimulate ovarian oxytocin output in sheep. J Endocrinol 122: 147-159.

Kotwica J (**1980**) Mechanism of prostaglandin $F_2\alpha$ penetration from the horn of the uterus to the ovaries in pigs. J Reprod Fertil 59: 237-241.

Kiriyama M, Ushikubi F, Kobayashi T, Hirata M, Sugimoto Y, Narumiya S (**1997**) Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. Br J Pharmacol 122: 217-224.

Krzymowski T, Czarnocki J, Koziorowski M, Kotwica J, Stefańczyk-Krzymowska S (**1986**) Counter current transfer of 3H-$PGF_2\alpha$ in the mesometrium: a possible mechanism for prevention of luteal regression. Anim Reprod Sci 11: 259-272.

Krzymowski T, Kotwica J, Stefańczyk-Krzymowska S, Czarnocki J, Koziorowski M (**1987**) Prostaglandin $F_2\alpha$ back transfer from the mesometrium vasculature into the uterus of the gilt during early pregnancy and estrogen-induced pseudopregnancy. Anim Reprod Sci 13: 199-210.

Maigaard S, Forman A, Andersson KE (**1985**) Differences in contractile activation between human myometrium and intramyometrial arteries. Acta Physiol Scand 124: 371-379.

Maigaard S, Forman A, Andersson KE (**1985**a) Different responses to prostaglandin F_2 alpha and E_2 in human extra- and intramyometrial arteries. Prostaglandins 30: 599-607.

Pusateri AE, Rothschild MF, Warner CM, Ford SP (**1990**) Changes in morphology, cell number, cell size and cellular estrogen content of individual littermate pig conceptuses on days 9 to 13 of gestation. J Anim Sci 68: 3727-3735.

Schmitz T, Levine BA, Nathanielsz PW (**2006**) Localization and steroid regulation of prostaglandin E_2 receptor protein expression in ovine cervix. Reproduction 131: 743--750.

Skipor J, Kowalik A, Stefańczyk-Krzymowska S (**2007**) Luteinising hormone attenuates the vascular response to norepinephrine. Acta Vet Hung 55: 251-257.

Stefańczyk-Krzymowska S, Chłopek J, Grzegorzewski W, Radomski M (**2005**) Local transfer of prostaglandin E_2 into the ovary and its retrograde transfer into the uterus in early pregnant sows. Exp Physiol 90: 807-814.

Stefańczyk-Krzymowska S, Grzegorzewski W, Skipor J, Wąsowska B, Krzymowski T (**1997**) Involvement of adrenoceptors in the ovarian vascular pedicle in the regulation of counter current transfer of steroid hormones to the arterial blood supplying the oviduct and uterus of pigs. Br J Pharmacol 120: 763-768.

Stefańczyk-Krzymowska S, Krzymowski T, Einer-Jensen N, Kamiński T, Kotwica J (**1990**) Local transfer of prostaglandin $F_2\alpha$ from the uterine lumen into the venous and arterial blood and into the uterine, mesometrial and ovarian tissue on day 18 of pregnancy in the pig. Anim Reprod Sci 23: 223-235.

Stefańczyk-Krzymowska S, Krzymowski T, Wąsowska B, Chłopek J (**2002**) Retrograde transfer of ovarian steroid hormones to the ovary in the porcine periovarian vascular complex. Exp Physiol 87: 361-371.

Stefańczyk-Krzymowska S, Skipor J, Grzegorzewski W, Wąsowska B, Krzymowski T (**1994**) Local increase of steroid hormone concentrations in blood supplying the uterus and oviduct in anaesthetized and conscious gilts. Anim Reprod Sci 37: 35-41.

Stefańczyk-Krzymowska S, Szafrańska B, Kamiński T, Krzymowski T (**1994a**) Total content of prostaglandin $F_2\alpha$ in the endometrium and myometrium from various sections of the porcine uterine horn during the estrous cycle. Prostaglandins 48: 99-108.

Stefańczyk-Krzymowska S, Wąsowska B, Chłopek J, Gilun P, Grzegorzewski W, Radomski M (**2006**) Retrograde and local destination transfer of uterine prostaglandin E_2 in early pregnant sow and its physiological consequences. Prostaglandins Other Lipid Mediat 81: 71-79.

Wąsowska B, Stefańczyk-Krzymowska S (**2006**) Retrograde transfer of testosterone to the porcine ovary in follicular and luteal phase of the estrous cycle. Reprod Biol 6: 149-159.

White RE (**2002**) Estrogen and vascular function. Vascul Pharmacol 38: 73-80.