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A Study on the Effects of Inhibition of Prostaglandin Biosynthesis with Flunixin Meglumine and Later Administration of Prostaglandin $F_{2\alpha}$ on the Intraluminal Pressure Variations in the Isthmus of the Oviduct in Unrestrained Gilts

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Pettersson, A., S. Einarsson and H. Kindahl: A study of the effects of inhibition of prostaglandin biosynthesis with flunixin meglumine and later administration of prostaglandin $F_{2\alpha}$ on the intraluminal pressure variations in the isthmus of the oviduct in unrestrained gilts. Acta vet. scand. 1993, 34, 125-131. – Three gilts were each equipped with 2 ultra-miniature pressure sensors, placed at 2 different points along the same isthmus of the oviduct. Following base recordings of isthmic intraluminal pressure, the gilts were treated with 2.2 mg flunixin meglumine (FM) per kg body weight. After FM treatment, the peripheral plasma levels of 15-ketodihydro-$PGF_{2\alpha}$, the major metabolite of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), decreased within 30 min. The frequency of the phasic pressure fluctuations in the isthmus of the oviduct decreased after FM treatment. Exogenous administration of $PGF_{2\alpha}$ increased the peripheral plasma levels of 15-ketodihydro-$PGF_{2\alpha}$. When administered at a dose of 0.1 mg, $PGF_{2\alpha}$ produced an increase in the frequency of the phasic pressure fluctuations in the oviductal isthmus. When the $PGF_{2\alpha}$ dose was increased to 0.5 mg, a marked increase in the base and total pressures was seen in addition to the increase in the frequency of the phasic pressure fluctuations.

porcine; oviductal isthmus.

Introduction

The recording of intraluminal pressure variations can be used as an indirect method for studying muscle contractions in tubular organs.

Cyclic variations of the intraluminal pressure in the porcine oviductal isthmus have been demonstrated in vitro (*Rodriguez-Martinez et al.* 1982 a) and in vivo both in anaesthetized (*Rodriguez-Martinez et al.* 1982 b) and unrestrained gilts (*Pettersson* 1991). In unrestrained gilts, intraluminal pressure tended to increase through prooestrus and the first day of standing oestrus (day 1), after which it tended to decrease, with low pressures recorded on days 3 and 4 (*Pettersson* 1991). The regulatory mechanisms of these cyclic variations in intraluminal pressure are still not fully understood. Exogenous administered prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has a contractile effect on the porcine myosalpinx, both in vitro, and in anaesthetized gilts, in vivo (*Rodriguez-Martinez & Einarsson* 1985). The purpose of this study was to see if the intraluminal pressure in the isthmus of the oviduct in unrestrained gilts could be affected by a decreased endogenous prostaglandin production brought about by treating the gilts with the non-steroidal anti-inflammatory drug flunixin meglumine (FM) and if administration of $PGF_{2\alpha}$ could reverse any such effect.

Materials and methods

Animals

Three cycling mature Swedish Yorkshire gilts were used as test animals. They were housed indoors in a conventional stable and tested for signs of standing oestrus, in the presence of a fertile boar, by experienced personnel. Each animal was used only once. At least 1 week prior to initiating the study, the gilts were equipped with permanent jugular vein catheters (*Rodriguez & Kunavongkrit* 1983) so that blood samples could be taken without disturbing the animal. Special care was taken to accustom the gilts to human handling, in order to avoid stress during the test period.
The gilts were either in prooestrus or on the first day of oestrus during the test periods.

Experimental Design

Two ultra-miniature pressure sensors (PR-249, Millar Instr. USA), placed at 2 different points along the same isthmus of the oviduct were used for monitoring intraluminal pressure variations (*Henriksson et al.* 1987). The atmospheric pressure on the day of operation was used as a reference value for the entire test period. Intraluminal pressure recordings were started 9:00 a.m. on the following morning. After an initial 60 min period of intraluminal pressure recordings, the gilts were given an intravenous injection of FM (Finadyne®, Schering Corporation, Kenilworth, USA) at the dose of 2.2 mg/kg body weight. The intraluminal pressure recordings were discontinued for 60 min following the FM treatment after which the recordings were resumed and continued for 60 min. Up to this point, blood samples were collected every 30 min. The animals were then injected with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Dinolytic®, Upjohn Limited, Crawley, England) intravenously, twice, with a 60 min interval separating the injections. Intraluminal pressure was recorded continuously until 60 min had elapsed after the last injection. Gilts nos. 2 and 3 were first treated with 0.1 mg and 60 min later, with 0.5 mg PGF2_, while gilt no. 1 was treated first with the higher dose and 60 min later with the lower dose. Blood samples were collected at 5 and 10 min after receiving $PGF_{2\alpha}$ and then every 10 min until 60 min had elapsed from the administration of $PGF_{2\alpha}$. Prior to removing the pressure sensors, it was confirmed that the pressure sensors had retained their proper positions. Sections were removed from both oviducts from each gilt for histological examination under a light microscope.

Blood Samples

All blood samples were collected into heparinized Vacutainer® tubes (Becton and Dickinson, USA) and immediately centrifuged. Plasma was removed and stored at -20°C until analysed by radioimmunoassay for concentrations of progesterone (*Bosu et al.* 1976), oestradiol-17ß (*Boilert et al.* 1973) and 15-ketodihydroprostaglandin $F_{2\alpha}$ (*Granström & Kindahl* 1982). The methods used have earlier been validated for the porcine species (*Kunavongkrit at al.* 1983).

Calculations

When calculating the results, all calculations were based on the recordings obtained from the distal pressure sensor. The proximal pressure sensor was used for determining the propagation direction of outburst of increased

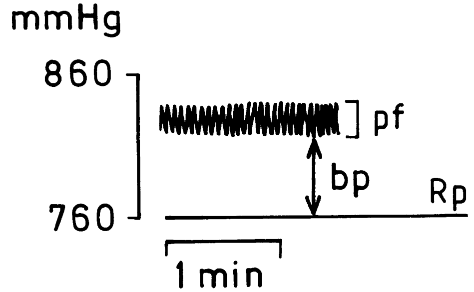

Fig. 1. Example of the phasic pressure fluctuations in the isthmus. Rp: atmospheric pressure, bp: base pressure, pf: phasic pressure fluctuations.

intraluminal pressure. The base line was set equivalent to the atmospheric pressure of the operation day. Intraluminal pressure could be described as being composed of a base pressure upon which phasic pressure fluctuations were superimposed (Fig. 1). The phasic pressure fluctuations could be arranged in a wavy, irregular or regular pattern with stable resting pressures and amplitudes. Outbursts of increased pressure were defined as marked increases in intraluminal pressure, where the lowest resting pressure of the phasic pressure fluctuations at the peak of the outburst was greater than the total pressure of the registration period. The base pressure was defined as equivalent to the lowest resting pressure during a 5 or 10 min period. The total pressure was derived by adding the mean amplitude of the phasic pressure fluctuations determined for 2 nonconsecutive min when the gilt was relatively still, to the base pressure. The frequency of the pressure fluctuations was also determined. The base pressure, the total pressure and the frequency of the phasic pressure fluctuations were determined for 10 min periods and two 5 min periods after each $PGF_{2\alpha}$ injection, in connection with each blood sample.

Results

Flunixin treatment had a clear inhibitory effect on the circulating levels of 15-ketodihydro-$PGF_{2\alpha}$ (Table 2). In all 3 gilts, the frequencies of the phasic pressure fluctuations decreased after treatment with FM. The total pressure recorded in gilt no. 3 was also lower after FM treatment (Table 1). The patterns in which the phasic pressure fluctuations were arranged did not change after FM treatment. No outburst of increased pressure occurred prior to or after FM treatment.

Both injections of $PGF_{2\alpha}$ resulted in a large increase in the prostaglandin metabolite concentrations (Table 2).

Treatment with $PGF_{2\alpha}$ resulted in an increase in the frequency of the phasic pressure fluctuations. When the high dose of $PGF_{2\alpha}$ was used, a transient dramatic effect on the base pressure and the total pressure in the oviductal isthmus was seen (see Table 2). This increase in intraluminal pressure was recorded simultaneously by both pressure sensors.

No adhesions were observed when inspecting the oviducts after completion of the pressure recordings. The pressure sensors had retained their proper positions. No differences were seen when histological preparations from both oviducts of each animal were compared under a light microscope.

Discussion

The results from this study indicate that endogenous prostaglandins are involved in maintaining the intraluminal pressure in the oviduct during prooestrus and day 1 of the oestrous cycle.

Exogenous administration of drugs often results in unphysiological drug tissue levels. The observed effects might therefore be a result of the dose used, rather than of the drug's physiological effect. By studying the effect of a de-

Table 1. The effect of i.v. administration of 2.2 mg/kg body weight of flunixin meglumine (FM) and later administration of prostaglandin $F_{2\alpha}$ on the base pressure (B) in mmHg, total pressure (TP) in mmHg and frequency (F) of intraluminal pressure fluctuations in the isthmus of the oviduct in unrestrained gilts. SD is the standard deviation of the amplitudes, expressed in mmHg, of the phasic pressure fluctuations included in each total pressure.

	Minutes																			
	0-10	20-30	50-60	120-130	140-150	170-180	180-185	185-190	190-200	200-210	210-220	220-230	230-240	240-245	245-250	250-260	260-270	270-280	280-290	290-300
Gilt 1				FM ↓			$PGF_{2\alpha}$ (0.5 mg) ↓							$PGF_{2\alpha}$ (0.1 mg) ↓						
B	752	772	772	762	762	752	822	782	782	772	762	752	762	762	772	762	752	752	752	762
TP	844	842	841	824	842	811	872	880	870	847	840	833	828	841	848	845	833	830	819	846
(SD)	(15)	(19)	(18)	(13)	(21)	(14)	(12)	(29)	(24)	(18)	(23)	(12)	(14)	(26)	(23)	(11)	(19)	(25)	(23)	(17)
F	36	36	37	31	31	31	49	40	36	34	34	30	32	41	36	32	35	33	32	34
Gilt 2				FM ↓			$PGF_{2\alpha}$ (0.1 mg) ↓							$PGF_{2\alpha}$ (0.5 mg) ↓						
B	750	750	750	760	760	760	770	760	750	750	750	750	750	800	790	780	790	780	780	790
TP	877	863	870	832	796	850	869	845	838	855	825	853	833	950	932	968	895	910	921	905
(SD)	(27)	(28)	(20)	(26)	(9)	(22)	(26)	(17)	(24)	(16)	(9)	(18)	(29)	(15)	(38)	(32)	(26)	(21)	(19)	(26)
F	27	26	25	19	18	17	28	25	22	20	19	20	19	28	23	26	24	22	23	23
Gilt 3				FM ↓			$PGF_{2\alpha}$ (0.1 mg) ↓							$PGF_{2\alpha}$ (0.5 mg) ↓						
B	785	785	775	765	775	765	765	765	765	765	765	765	765	795	805	775	765	775	765	765
TP	888	868	879	820	807	803	815	810	797	797	799	797	797	867	856	829	811	830	813	801
(SD)	(20)	(15)	(10)	(15)	(10)	(16)	(8)	(10)	(12)	(14)	(20)	(18)	(16)	(20)	(13)	(10)	(11)	(8)	(9)	(6)
F	38	36	34	17	12	10	28	23	19	18	20	19	17	47	41	35	27	22	20	2

Table 2. Progesterone (Prog), oestradiol-17ß (E2) and 15-ketodihydro-$PGF_{2\alpha}$ (PG) levels in peripheral circulation before and after flunixin meglumine (FM) and later $PGF_{2\alpha}$ treatment. Progesterone levels are expressed in nmol/l while oestradiol-17ß and 15-ketodihydro-$PGF_{2\alpha}$ levels are expressed in pmol/l.

	Minutes																							
	0	30	60		90	120	150	180		185	190	200	210	220	230	240		245	250	260	270	280	290	300
Gilt 1				FM ↓					$PGF_{2\alpha}$ (0.5 mg) ↓								$PGF_{2\alpha}$ (0.1 mg) ↓							
Prog	1.8	1.5	2.0		1.7	2.1	2.3	2.4		1.8	0	1.7	2.2	2.7	1.9	2.0		2.6	2.5	2.6	2.4	2.6	2.1	0.2
E2	46	45	42		40	45	37	32		40	37	47	49	49	52	38		31	30	31	30	30	42	35
PG	329	300	317		105	<75	<75	<75		26.5×10^3	13.6×10^3	5.1×10^3	2794	1840	1276	707		5.7×10^3	>1200	1351	792	742	558	404
Gilt 2				FM ↓					$PGF_{2\alpha}$ (0.1 mg) ↓								$PGF2_{\alpha}$ (0.5 mg) ↓							
Prog	0	1.5	0		2.9	2.1	2.1	2.1		0	1.6	0	1.0	2.2	1.9	0.7		2.1	2.5	4.4	31	2.5	4.1	3.0
E2	49	52	34		56	39	47	51		60	52	55	59	60	61	58		64	53	51	5.1	59	59	53
PG	236	244	152		<75	<75	<75	<75		5.8×10^3	2634	729	472	278	212	100		47.2×10^3	21.3×10^3	6.8×10^3	2769	1669	1319	1084
Gilt 3				FM ↓					$PGF_{2\alpha}$ (0.1 mg) ↓								$PGF_{2\alpha}$ (0.5 mg) ↓							
Prog	3.0	3.1	3.9		3.7	4.2	4.2	3.0		4.5	3.5	3.1	3.1	3.4	3.8	2.1		2.8	4.1	4.2	4.1	2.3	3.2	2.8
E2	53	58	55		56	66	52	49		58	69	78	67	65	54	67		63	59	71	73	69	55	57
PG	154	517	150		<75	<75	<75	<75		3705	2971	1140	640	323	295	117		24.8×10^3	11×10^3	3220	2296	1471	1274	872

creased endogenous production of prostaglandins on the intraluminal pressure in the oviductal isthmus, possible pharmacological effects of $PGF_{2\alpha}$ could be avoided.

Flunixin has been shown to be a potent inhibitor of prostaglandin synthesis in the porcine species (*Odensvik et al.* 1989). In the present study, 15-ketodihydro-$PGF_{2\alpha}$ levels in peripheral circulation had decreased within 30 min after FM treatment. This is in line with the results obtained by *Odensvik et al.* (1989), who found that 15-ketodihydro-$PGF_{2\alpha}$ levels in peripheral circulation decreased within 20 min after FM treatment. Further, low levels of 15-ketodihydro-$PGF_{2\alpha}$ were maintained for 5-6 h (*Odensvik et al.* 1989). It is likely that FM treatment affects the biosynthesis of prostaglandins in all tissues, including that which occurs in the oviduct. It has been shown that $PGF_{2\alpha}$ when administered both in vitro and in vivo in anaesthetized gilts stimulates oviductal contractility (*Rodriguez-Martinez & Einarsson* 1985). Furthermore, the decrease in the frequency of the phasic pressure fluctuations seen after FM treatment in the present study, indicates that the endogenous prostaglandin biosynthesis is involved in maintaining the intraluminal pressure in the isthmus of the oviduct during the period studied. During the periovulatory period, when intraluminal pressure in the oviductal isthmus is high (*Pettersson* 1991), high levels of $PGF_{2\alpha}$ have been found in the porcine oviductal fluid (*Rodriguez-Martinez et al.* 1983). The prostaglandins produced in the oviduct may have a stimulating effect on the myosalpinx during this period (*Rodriguez-Martinez et al.* 1983). Recognizing the need for further studies, it is possible that the decrease in the total pressure seen in the isthmus on day 1 (gilt no. 3) may be a result of decreased levels of prostaglandins in the oviductal fluid. The lack of dramatic effects on the base and total pressures after FM treatment in gilts nos. 1 and 2 was not surprising since other factors, including the rich adrenergic innervation of the isthmus (*Rodriguez-Martinez et al.* 1982 c), are involved in maintaining oviductal intraluminal pressure.

In the present study, treatment with $PGF_{2\alpha}$ consistently increased the frequency of the phasic pressure fluctuations in an apparantly dose-dependant manner. The higher dose of 0.5 mg also led to a marked increase in the base and total pressures. It would seem that 0.1 mg $PGF_{2\alpha}$ had a more physiological effect on the oviduct while a cramp-like condition was induced when 0.5 mg was administered.

Conclusion

Flunixin meglumine treatment resulted in a marked decrease in circulating levels of 15-ketodihydro-$PGF_{2\alpha}$. The decrease in endogenous prostaglandins resulted in a decreased frequency of the phasic pressure fluctuations in the isthmus of the porcine oviduct. Exogenous administration of $PGF_{2\alpha}$ had the reverse effect. The results from this study support the theory that the endogenous prostaglandins are involved in maintaining the intraluminal pressure in the porcine isthmus of the oviduct during prooestrus and oestrus.

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Sammanfattning

Effekten av prostaglandinsynteshämning med flunixin meglumin med efterföljande administrering av prostaglandin $F_{2\alpha}$på intraluminella tryckvariationer i istmusdelen av äggledaren hos gris.

Tre gyltor utrustades med 2 tryckmätare placerade på 2 olika punkter längs samma äggledares isthmus. Efter en inledande period med mätningar av det intraluminella trycket behandlades gyltorna med 2,2 mg flunixin meglumine (FM) per kg kroppsvikt. Blodplasmanivåerna av 15-ketodihydro-$PGF_{2\alpha}$ i perifera cirkulationen sjönk inom 30 minuter efter FM behandlingen. Frekvensen av de fasiska tryckförändringarna sjönk likaså efter FM behandlingen. En kraftig stegring av blodplasmanivåerna av 15-ketodihydro-$PG_{2\alpha}$ påvisades efter exogen tillförsel av PGF2_. Behandlingen med 0,1 mg $PGF_{2\alpha}$ resulterade i en ökad frekvens fasiska tryckfluktationer, medan 0,5 mg $PGF_{2\alpha}$ resulterade i en ökning av såväl basala trycket, totala trycket som av frekvensen fasiska tryckfluktationer.

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