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The Effect of Induced Standing Reflex, Cervical Stimulation and Insemination on Intraluminal Pressure Variations in the Isthmus of the Oviduct in Unrestrained Gilts

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Pettersson, A.: The effect of induced standing reflex, cervical stimulation and insemination on intraluminal pressure variations in the isthmus of the oviduct in unrestrained gilts. Acta vet. scand. 1993, 34, 117-124. – Four gilts were each equipped with 2 ultra-miniature pressure sensors, placed at 2 different points along the same isthmus of the oviduct, on the morning of the first day of standing oestrus (Day 1). Intraluminal pressure recordings were started the same afternoon. After an initial recording period, intraluminal pressure was recorded while the gilts showed standing oestrus and during cervical stimulation followed by insemination with either 100 ml saline or 100 ml boar semen. Monitoring of the pressure variations in the isthmus was continued for up to 6.5 h after the last insemination. Blood samples for monitoring oestradiol-17ß, progesterone and 15-ketodihydroprostaglandin $F_{2\alpha}$ were collected before and after each manipulation of the gilt and every 30 min during the rest of the test period. None of the above manipulations had any consistent effect on the intraluminal pressure in the porcine isthmus, although, a clear 15-ketodihydroprostaglandin $F_{2\alpha}$ peak could be seen after insemination with boar semen.

oviductal isthmus; porcine; prostaglandin $F_{2\alpha}$

Introduction

Under natural conditions gilts showing signs of standing oestrus are mated and a large volume of semen is propelled directly into the uterus. Although spermatozoa can be found in the oviducts shortly after mating (*First et al.* 1968), they are mainly limited to the uterotubal junction (UTJ) and the caudal portion of the oviductal isthmus (*Hunter & Léglise* 1971, *Hunter* 1975, *Viring et al.* 1980, *Fléchon & Hunter* 1981). Evidence has been presented suggesting that the porcine isthmus and UTJ play an active role in limiting the ascent of spermatozoa to the ampullary-isthmic junction (AIJ), where fertilization occurs (*Hunter & Léglise* 1971). The oedematous condition of the luminal folds and the processes of the UTJ (*Andersen* 1928, *Hunter* 1981) together with the high intraluminal pressure measured in the isthmus of the oviduct during standing oestrus (*Pettersson* 1991) may be important factors in the regulation of sperm admission to the oviduct and the establishment of a sperm reservoir. It is, however, possible that various stimuli involved in the process of mating and the gametes themselves may affect the conditions in the oviduct. In this study, the effects of induced standing reflex, cervical stim-

ulation and insemination, with either 100 ml saline, or 100 ml fresh semen from a fertile boar on the intraluminal pressure in the oviductal isthmus in unrestrained gilts were examined.

Materials and methods

Animals

Four mature cycling Swedish Yorkshire gilts were used as test animals. They were housed indoors in a conventional stable and checked for standing oestrus twice daily, in the presence of a mature boar, by experienced personnel. The gilts had shown at least 1 oestrus prior to use in the experiment. Each gilt was used only once. Special care was taken to accustom the gilts to human handling, in order to minimize stress during the test period.

Surgery

The gilts were equipped with chronic jugular vein catheters (*Rodriguez & Kunavongkrit* 1983) 1 week before expected oestrus. Blood samples could thereby be collected without disturbing the animal. Early, on the morning of the first day of standing oestrus (Day 1 of the oestrous cycle), 2 ultra-miniature pressure sensors (PR-249, Millar Instr., USA), each located at the distal end of a 140 cm long dacron catheter with an electrical connector at its proximal end, were passed from the lumbar back subcutaneously, and placed at 2 different points along the same isthmus. The electrical connectors were placed in a canvas bag sutured to the animal's back. A 5 % aqueous solution of thiopentone sodium (Pentothal Sodium, Abbott) was used for inducing general anaesthesia while inhalation of halothane (Halothan, Hoechst, Germany) was used for maintaining anaesthesia. This method has previously been described in detail (*Henriksson et al.* 1987). The gilts were then returned to the stable.

Experimental Design

The gilts were allowed to regain consciousness and the experiment was started late, on the afternoon of the same day. During an initial period of 30 min, intraluminal pressure recordings were made, prior to exposing the gilts to a boar. Gilt no. 1 was moved to a farrowing crate positioned in front of the boar's pen, while the remaining 3 gilts stayed in their own pens and the boar was instead moved into an adjacent pen with rather large holes in the wall, through which the boar was able to nuzzle the gilts but not mount them. The standing reflex was induced manually in the presence of the boar. Intraluminal pressure was monitored continuously. After 5 to 10 min, a sterile rubber insemination catheter was introduced into the cervix while the gilt exhibited the standing reflex. The cervix was stimulated for 5 to 10 minutes. This procedure was repeated in gilt no. 1 after a 30 min period of intraluminal pressure recordings without the presence of the boar. After the second period of cervix stimulation, she was directly inseminated with 100 ml body temperature saline solution. Gilt no. 2 was inseminated with 100 ml saline after the first period of cervix stimulation. The boar was then removed and after 60 min, during which time intraluminal pressure was continuously recorded, the boar was returned and the process of inducing standing reflex and cervical stimulation was repeated. The gilt was then inseminated with 100 ml filtered fresh semen, manually collected from a fertile boar. The remaining 2 gilts were only induced to show the standing reflex once and each cervix stimulated once after which they were directly inseminated with 100 ml fresh filtered boar semen from a fertile boar. The insemination doses used contained between 21 and 36×10^9 spermatozoa. The oestrogen content in the inseminates varied. The oestradiol-17ß levels ranged from

224 pmol/l to 584 pmol/l while oestrone sulphate levels ranged from 0.3 nmol/l to 5.6 nmol/l. Intraluminal pressure recordings were continued for up to 6.5 h after the last insemination. The pressure sensors were later removed by laparotomy. The proper positions of the pressure sensors were confirmed and the ovary and oviduct carefully inspected. After slaughter, which occurred at a later date, sections from both oviducts of each gilt were removed. Histological preparations of the oviducts were later compared under a light microscope.

Blood samples and analytical methods

Blood samples were collected into heparinized Vacutainer® tubes (Becton and Dickinson, Rutherford, USA) every 30 min and also before and after each manipulation of the gilt. The blood samples were immediately centrifuged and the plasma withdrawn and stored at -20°C until hormonal analysis could be made. The blood samples were later analysed for levels of the main prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) metabolite, 15-ketodihydroprostaglandin $F_{2\alpha}$ (*Granström & Kindahl* 1982), oestradiol-17ß (*Boilert et al.* 1973), and progesterone (*Bosu et. al.* 1976), using radioimmunoassays. Oestrone sulphate was determined, using a modified version of the radioimmunoassay described by *Kindahl et al.* (1982). Oestradiol-17ß and oestrone sulphate levels were also determined in the semen, used in the experiment utilizing the same techniques. The methods used for progesterone, oestradiol-17ß and 15-ketodihydro-$PGF_{2\alpha}$ have earlier been validated for the porcine species (*Kunavongkrit et al.* 1983).

Calculations

Oviductal intraluminal pressure can be described as being composed of phasic pressure fluctuations superimposed upon a base pressure (Fig. 1). The phasic pressure fluctuations can be arranged in a wavy, irregular or regular pattern with stable resting pressures and amplitudes. Outbursts of increased intraluminal 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. Prior to insertion, the pressure sensors were calibrated to the atmospheric pressure of the operation day. This pressure was used as a reference during the entire test period. The base pressure was defined as the lowest resting pressure of the phasic pressure variations during 5 or 10 min periods. The total pressures for these periods were derived by adding the sum of the mean amplitudes of the phasic pressure fluctuations during 2 nonconsecutive min to the base pressure. The frequency of the phasic pressure fluctuations was also determined. Calculations were based on the recordings obtained from the distal pressure sensor. The proximal pressure sensor was used for determining the propagation direction of outbursts of increased intraluminal pressure.

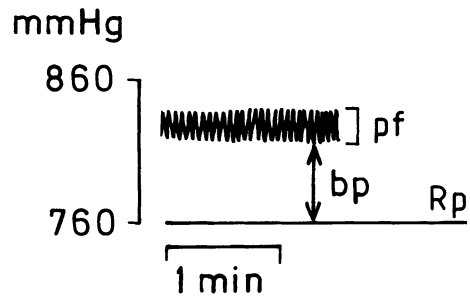

Fig. 1. Example of the phasic pressure fluctuations in the isthmus. Rp: atmospheric pressure, bp: base pressure, pf: phasic pressure fluctuations.

Results

As seen in Table 1, none of the stimuli to which the gilts were exposed resulted in any consistent alteration of the intraluminal pressure parameters recorded. The pattern in which the phasic pressure fluctuations were arranged was not affected by any of the treatments. One outburst of increased intraluminal pressure was seen in gilt no. 1 about 45 min after insemination with saline. This outburst occurred simultanously at both points of registration. The 2 gilts (gilts nos. 3 and 4) inseminated with boar semen had a 15-ketodihydro-$PGF_{2\alpha}$ peak which started approximately 220 and 255 min respectively after insemination. The levels of 15-ketodihydro-$PGF_{2\alpha}$ in gilt no. 1, who had been inseminated with saline only, did not increase after insemination. Gilt no. 2, having been inseminated with both saline and semen, showed a tendency for a peak towards the end of the registration about 250 min after insemination with boar semen (Table 2). These prostaglandin peaks were not followed by any consistent change in the intraluminal pressure parameters. Oestradiol-17ß and oestrone sulphate levels in the peripheral circulation remained uneffected by the insemination procedures.

No adhesions were seen involving the oviduct when removing the pressure sensors on the following day. The positions of the pressure sensors had not changed during the test period. All gilts had ovulated. The histological examination did not reveal any damage to the oviduct.

Discussion

Judging from the results obtained in this study, it does not seem as though the processes of induced standing reflex or cervical stimulation have any consistent effect on the intraluminal pressure in the isthmus of the oviduct in unrestrained gilts. These findings are in line with the results obtained by *Bower* (1974), who found that external stimuli did not enhance uterine contractions in conscious pigs.

Mechanical stimulation of the cow's reproductive organs has been shown to stimulate the release of oxytocin (*Schams et al.* 1982). Although exogenous administration of oxytocin in anaesthetized gilts has been shown to increase oviductal activity during oestrus (*Zerobin & Spörri* 1972), no consistent response to the mechanical stimulation of the cervix was seen in the present study. One explanation for this might be that the pig does not always respond to mechanical stimulation of the reproductive organs by releasing oxytocin, as shown by *Claus* (1990). Further, the pharmacological and physiological effects of oxytocin on the oviduct may differ.

Uterine contractility is high during oestrus (*Zerobin & Spörri* 1972, *Bower* 1974, *Claus et al.* 1989). Further, myometrial activity can be enhanced by insemination both in vivo in anaesthetized sows (*Zerobin & Spörri* 1972) and in conscious pigs (*Bower* 1974). Uterine contractions in association with mating are thought to be actively involved in the transport of spermatozoa through the uterus to the UTJ (*Hunter* 1973). In the present study, however, no consistent effect of the insemination processes on the intraluminal pressure in the oviductal isthmus could be seen.

Claus et al. (1987) have suggested that the high oestrogen concentration in boar semen stimulates the uterus to synthesize, in particular, $PGF_{2\alpha}$ which in turn enhances uterus contractions. *Claus et al.* (1990) found that after mating, 15-ketodihydro-$PGF_{2\alpha}$ concentrations showed a biphasic pattern in peripheral plasma, which was also reflected by the oestradiol profile. Since half of the seminal oestrogens are bound to the spermatozoa (*Claus et al.* 1985), the biphasic pattern was explained

Table 1. The base pressure (BP), total pressure (TP) in mmHg, and frequency (F) of the phasic pressure fluctuations before, during and after induced standing reflex (S), cervical stimulation (C) and insemination with 100 ml saline (I) or 100 ml boar semen (IB). SD is the standard deviation of the amplitudes, expressed in mmHg, of the phasic pressure fluctuations included in each total pressure.

		Gilt 1						Gilt 2			
Min	Stim.*	B	TP	(SD)	F	Min	Stim.	B	TP	(SD)	F
0 - 10		790	870	(18)	37	0 - 10		765	833	(21)	38
10 - 20		780	883	(18)	36	20 - 30		775	835	(23)	41
55 - 60	S	800	887	(10)	37	35 - 40	S	775	842	(8)	43
60 - 70	SC	790	876	(15)	37	45 - 50	SC	785	862	(20)	45
90 - 100		780	845	(10)	36	55 - 60	SCI	765	849	(24)	44
120 - 125	SC	780	862	(30)	38	80 - 90		765	819	(21)	40
125 - 130	SCI	780	853	(21)	38	110 - 120		765	841	(16)	39
130 - 140		770	849	(13)	34	120 - 125	S	765	826	(7)	44
160 - 170		770	834	(16)	34	130 - 135	SC	765	823	(19)	43
190 - 200		780	876	(18)	34	140 - 145	SCIB	765	815	(20)	39
220 - 230		770	841	(19)	33	150 - 160		775	815	(19)	37
250 - 260		770	840	(40)	37	170 - 180		765	814	(18)	41
280 - 290		780	873	(40)	35	200 - 210		765	822	(16)	40
310 - 320		780	862	(16)	35	230 - 240		765	817	(25)	41
340 - 350		790	892	(28)	34	260 - 270		765	827	(22)	40
370 - 380		790	892	(26)	33	290 - 300		775	814	(18)	40
						320 - 330		765	809	(15)	42
						350 - 360		765	835	(21)	43
						380 - 390		765	830	(18)	41
						410 - 420		775	848	(16)	44

		Gilt 3						Gilt 4			
Min	Stim.	B	TP	(SD)	F	Min	Stim.	B	TP	(SD)	F
0 - 10		798	886	(18)	41	0 - 10		806	921	(22)	44
25 - 30		788	871	(18)	34	20 - 30		816	942	(22)	45
35 - 40	S	788	848	(25)	40	40 - 45	S	806	903	(32)	49
45 - 50	SC	788	868	(20)	40	50 - 55	SC	806	929	(24)	45
55 - 60	SCIB	788	846	(27)	38	55 - 60	SCIB	816	932	(26)	44
60 - 65	C	778	848	(21)	39	65 - 70		826	937	(17)	45
90 - 100		778	840	(25)	35	95 - 105		806	939	(28)	43
120 - 130		768	832	(18)	36	125 - 135		806	938	(17)	38
150 - 160		778	839	(20)	33	155 - 165		806	923	(21)	38
180 - 190		778	856	(17)	35	185 - 195		806	924	(10)	42
210 - 220		778	842	(22)	36	215 - 225		806	932	(13)	43
240 - 250		778	847	(26)	36	245 - 255		796	903	(13)	43
270 - 280		776	881	(30)	36	275 - 285		786	910	(17)	40
300 - 310		776	861	(21)	40	305 - 315		786	894	(13)	41
330 - 340		776	882	(24)	38	335 - 345		786	896	(20)	42
360 - 370		788	848	(13)	38	365 - 375		806	912	(20)	43
390 - 400		788	851	(14)	37	395 - 405		796	914	(12)	43
420 - 430		798	894	(24)	36	425 - 435		786	897	(17)	43

* Stim. = method of stimulation.

Table 2. Peripheral plasma levels of oestradiol-17ß (E_2) and 15-ketodihydro-$PGF_{2\alpha}$ (PG) before and after inducing standing reflex (S), cervical stimulation (C) and insemination with saline (I) or boar semen (IB).

Gilt 1				Gilt 2			
Min	Stim.*	E_2 (pmol/l)	PG (pmol/l)	Min	Stim.	E_2 (pmol/l)	PG (pmol/l)
0		80	275	0		56	451
20		87	243	30		54	366
60	S	69	236	40	S	52	566
70	SC	49	233	50	SC	60	651
100		58	199	60	SCI	46	471
115		43	317	90		32	489
125	SC	33	221	120		34	405
130	SCI	50	276	125	S	46	464
140		-	-	135	SC	34	452
170		66	247	145	SCIB	32	420
200		63	205	160		-	-
230		60	161	180		34	313
260		59	256	210		36	303
290		71	212	240		41	364
320		57	207	270		25	361
350		55	222	300		35	328
380		64	177	330		31	308
				360		24	261
				390		26	554
				420		20	667

Gilt 3				Gilt 4			
Min	Stim.	E_2 (pmol/l)	PG (pmol/l)	Min	Stim.	E_2 (pmol/l)	PG (pmol/l)
0		66	575	0		27	1000
30		40	430	30		28	918
40	S	68	405	45	S	21	945
50	SC	64	405	55	SC	26	922
60	SCIB	46	405	60	SCIB	17	536
65	C	61	385	65		22	667
100		57	313	70		24	753
130		62	400	105		28	527
160		46	420	135		25	536
190		50	573	165		23	590
220		43	496	195		26	641
250		42	522	225		20	641
280		58	1156	255		20	521
310		43	1352	285		38	356
340		48	3376	315		43	616
370		41	2122	345		22	1488
400		39	1059	375		20	1032
430		59	988	405		20	835
				435		26	435

* Stim. = method of stimulation.

as a result of a delayed release of the sperm-bound oestrogens. The insemination procedures used in the present study did not lead to any peaks in the levels of oestradiol-17ß in peripheral circulation. The increase in the prostaglandin metabolite seen 220 or 255 min after insemination might be a result of the leukocyte invasion into the uterus, which is normally seen after mating (*Lovell & Getty* 1968). It is interesting to note that although exogenous administration of $PGF_{2\alpha}$ is known to stimulate contractility in the porcine oviduct, both in vitro and in vivo (*Rodriguez-Martinez & Einarsson* 1985), no increased activity was seen in the isthmus corresponding to the 15-ketodihydro-$PGF_{2\alpha}$ peaks. One possible explanation for the lack of effect might be that some or all of the prostaglandins resorbed from the uterus might not reach the oviduct, but instead are metabolized and thereby inactivated in the lung. It is also possible that a corresponding increase in PGE_2, which has a relaxing effect on the isthmus during oestrus (*Rodriguez-Martinez & Einarsson* 1985), may occur, which would counteract the effect of $PGF_{2\alpha}$ on the oviduct.

Conclusions

No consistent effect on the intraluminal pressure in the isthmus of the oviduct was seen following induced standing reflex, stimulation of the cervix and insemination of saline or boar semen in gilts.

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Sammanfattning

Effekten av stäreflexen, cervixstimulering och inseminering på intraluminella tryckvariationer i istmusdelen av äggledaren hos gris.

Fyra gyltor utrustades med 2 små tryckmätare placerade på 2 olika punkter inuti samma äggledares isthmus på morgonen dag 1 i brunstcykeln. Mätningar av intraluminella tryckförändringar påbörjades samma eftermiddag. Det intraluminella trycket registrerades före, under och efter utlösning av ståreflexen, stimulering av cervix och insemination med antingen 100 ml koksaltlösning eller helejakulat från en fertil galt. Tryckmätningar utfördes upp till 6,5 t efter inseminationen. Blodprov samlades var 30:e min samt efter varje manipulation med gyltorna. Blodproven analyserades på sitt innehåll av östradiol-17ß, östronsulfat, progesteron samt 15-ketodihydroprostaglandin $F_{2\alpha}$. Ingen av behandlingarna som gyltorna utsattes för resulterade i någon återkommande effekt på det intraluminella trycket i äggledarens isthmus. En ökning av 15-ketodihydroprostaglandin $F_{2\alpha}$ syntes dock cirka 4 t efter insemination med helejakulat.

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