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Poster

Impact of stress during oestrus on the oviductal sperm reservoir and on progesterone concentrations in the sow

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Aims

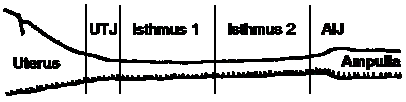
The objective of the study was to investigate if short-term stress in sows (simulated by injections of synthetic ACTH) during standing oestrus had an effect on the distribution of spermatozoa in the utero-tubal junction (UTJ) and isthmus and on the progesterone concentrations.

Introduction

"Stress" has been defined as the inability of an animal to cope with its environment (Dobson and Smith, 2000). Due to welfare considerations, some countries have begun introducing systems with group-housing of sows, the most common type in Sweden being where sows are allocated to groups immediately after weaning. In systems with group-housing, stress levels in a newly formed group of sows will be at its highest for approximately two days until a ranking order is established (Tsuma et al., 1996), and the effects of stress might even persist as long as two weeks after the actual regrouping event (Stookey and Gonyou, 1994). Even though systems involving group-housing of sows are in many ways very appealing, the effects of high stress levels during a short but critical phase of the reproductive cycle, e.g. resumption of ovarian activity, oestrus and ovulation, have yet to be clarified.

Materials and methods

Fourteen sows were monitored for ovulation using transrectal ultrasonography in two consecutive oestruses. The sows were fitted with jugular catheters and, from onset of the second oestrus, blood samples were collected every 2 h. In the 2nd oestrus, 7 sows were given ACTH every 2ndh, from the onset of standing oestrus until the sow ovulated (ACTH-group), whereas the other 7 sows remained as controls (C-group) and were given NaCl solution at corresponding times. The sows were artificially inseminated 16-18h before expected ovulation (as estimated from their first oestrus) with 10 billion fresh spermatozoa (70% progressive motile) pooled from two boars with proven fertility. Six hours after ovulation the sows were euthanased and the UTJ and oviducts were removed. The oviducts were thereafter divided in 3 adjacent sections consisting of; (i) the UTJ, (ii) the first, and (iii) the second isthmus segment. These segments were flushed to retrieve spermatozoa.



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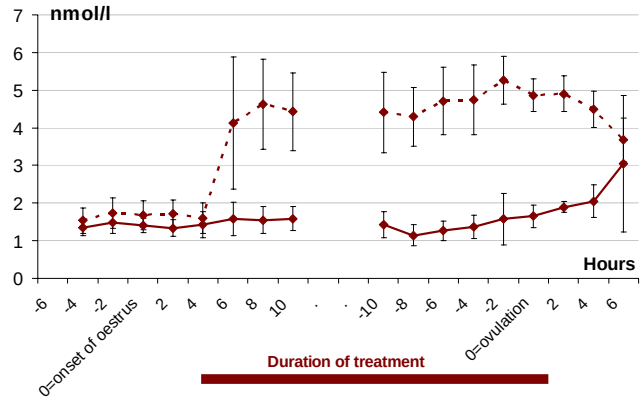
Results

The concentrations of cortisol in jugular blood of the ACTH-group sows during the time of ACTH-injections were significantly higher than of the C-group sows ($p<0.05$), as were the levels of progesterone ($p<0.001$).

The concentrations of progesterone during treatment
(mean ±SD, N=7 in each group, ¯=C-group, ---= ACTH-group)



There was a tendency for a larger number of spermatozoa among sows in the ACTH-group, especially in the isthmic segment adjacent to the AIJ.

Distribution of spermatozoa in the different segments of the oviduct (geometric means)

Oviductal segment	C-group	ACTH-group
UTJ	19102 a	26267 a
I1 (Isthmus 1)	136 b	582 b
I2 (Isthmus 2)	3 c	73 b

Values within columns followed by different letters differ significantly ($P<0.05$).

Discussion

In addition to higher concentrations of cortisol during treatment, sows in the ACTH-group also displayed a major elevation of basal progesterone concentrations in jugular blood, probably originating from the stimulation of the adrenal glands (Bolaños et al., 1997; Tsuma et al., 1998; Mwanza et al., 2000). The elevated progesterone might have had an impact on the functions of the uterus, oviduct (Day and Polge, 1968; Hunter, 1981; Gawronska et al., 2000), ovary (Close and Liptrap, 1975) and possibly also on the spermatozoa since progesterone has been reported to play a role in sperm capacitation (Barboni et al., 1995) and acrosome reaction (Sueldo et al., 1993; Melendrez et al., 1994).

Earlier studies on the distribution of spermatozoa in the genital tract of pigs at different times after pre-ovulatory insemination have indicated that the UTJ is likely to be populated within a few hours after insemination, and that this functional sperm reservoir is established and remains relatively constant from approximately 6 to 24 hours after insemination (Viring, 1980; Viring and Einarsson, 1981; Mburu et al., 1996; Rodriguez-Martinez, 2001). For this reason, two animals were excluded from the present study due to an interval of more than 30 hours between insemination and ovulation (one control and one treated sow). The present result coincides with

earlier studies, where the largest numbers of spermatozoa were always found in the UTJ and adjacent part of the isthmus, with sperm numbers gradually decreasing closer to the AIJ segment (Viring, 1980; Viring and Einarsson, 1981; Mburu et al., 1996). The UTJ is believed to act as a mechanical barrier, but might also possess other properties to prevent excessive colonisation of the oviduct and ensure a proper release of viable spermatozoa close to ovulation. The ACTH-group demonstrated tendencies for larger numbers of spermatozoa to be found in the UTJ and isthmus than the C-group (see table 3-1). The high levels of progesterone in the ACTH-group during insemination and up till ovulation may have affected the transport of spermatozoa in the oviduct. Day and Polge (1968) found that gilts injected with progesterone had a higher incidence of polyspermy. The mechanism for this is not known but progesterone might act through changes in the environment of the oviduct and/or changes in the spermatozoa themselves. Injections of progesterone reduce the oedema at the UTJ and in the longitudinal folds of the isthmus and might thereby facilitate sperm passage (Hunter, 1981). Recent studies have postulated that progesterone together with oestradiol cause relaxation of the porcine oviduct (Gawronska et al., 2000). Another possible explanation of these larger numbers of spermatozoa in the ACTH-group may be the $PGF_{2\alpha}$-metabolite peak following each ACTH injection (Razdan et al., 2002; Lang et al., unpublished results). Administration of $PGF_{2\alpha}$ results in an increased frequency and amplitude of contractions of both the uterus and oviduct (Edqvist et al., 1975; Rodriguez-Martinez & Einarsson, 1985; Pettersson et al., 1993; Mwanza et al., 2000), thereby facilitating the first phase of sperm transport. The uterus and oviduct stand under oestrogen stimulation before ovulation, and are therefore more sensitive to $PGF_{2\alpha}$, than during the non-oestrogen phase of the reproductive cycle.

Hunter (1981) and Mburu et al. (1996) provided interesting views of the distribution of boar spermatozoa within the porcine oviduct around ovulation. Their studies revealed that there is a sperm release and transport from the UTJ towards the AIJ around ovulation but, contrary to the views of Hunter (1981), the distribution is not bulk-wise, but more of a sequential nature (Mburu et al., 1996). The present results indicate a facilitated transport of spermatozoa in the ACTH-group, seemingly through the entire female reproductive tubular system, but with special reference to the area close to the AIJ. A facilitated transport together with elevated levels of progesterone may result in large numbers of spermatozoa at the fertilisation site, which might give rise to polyspermy (Hunter, 1991) which, being a lethal condition, will have a negative impact on fertility.

In this study, the sperm distribution was investigated, and to some extent the local environment in and around the isthmus after simulated stress from onset of standing oestrus until ovulation. Naturally, there might be other areas affected by simulated stress than those brought forth in this study, e.g. follicle development and oocyte quality.

Conclusion

In conclusion, simulated stress induced by injections of ACTH during standing oestrus results in elevated concentrations of progesterone. ACTH-injections also appeared to augment transport of spermatozoa through the female genital tract of pigs.

References

Barboni, B., Mattioli, M., Seren, E., 1995. Influence of progesterone on boar sperm capacitation. J Endocrinol. 144, 13-18.

Bolaños, J.M., Molina, J.R., Forsberg, M., 1997. Effect of blood sampling and administration of ACTH on cortisol and progesterone levels in ovariectomized zebu cows (Bos indicus). Acta Vet. Scand. 38, 1-7.

Close, R.W., Liptrap, R.M., 1975. Plasma progesterone levels in sows with induced cystic ovarian follicles. Res Vet Sci. 19, 28-34.

Day, B.N., Polge, C., 1968. Effects of progesterone on fertilisation and egg transport in the pig. J. Reprod. Fert. 17, 227-230.

Dobson, H., Smith, R.F., 2000. What is stress, and how does it affect reproduction? Anim. Reprod. Sci. 60-61, 743-752.

Edqvist, S., Einarsson, S., Gustafsson, B., Linde, C., Lindell, J-O., 1975. The in vitro and in vivo effects of prostaglandins E_1 and $F_{2\alpha}$ and of oxytocin on the tubular genital tract of ewes. Int. J. Fertil. 20, 234-238.

Gawronska, B., Stepien, A., Ziecik, A.J., 2000. Effect of estradiol and progesterone on oviductal LH-receptors and LH-dependent relaxation of the porcine oviduct. Theriogenlogy, 53, 659-672.

Hunter, R.H.F., 1981. Sperm transport and reservoirs in the pig oviduct in relation to the time of ovulation. J. Reprod. Fert. 63, 109-117.

Hunter, R.H.F., 1991. Oviduct function in pigs, with particular reference to the pathological condition of polyspermy. Mol. Reprod. Dev. 29, 385-391.

Mburu, J.N., Einarsson, S., Lundeheim, N., Rodriguez-Martinez, H., 1996. Distribution, number and membrane integrity of spermatozoa in the pig oviduct in relation to spontaneous ovulation. Anim. Reprod. Sci. 45, 109-121.

Melendrez, C.S., Meizel, S., Berger, T., 1994. Comparison of the ability of progesterone and heat solubilized porcine zona pellucida to initiate the porcine sperm acrosome reaction in vitro. Mol Reprod Dev. 39, 433-438.

Mwanza, A.M., Madej, A., Kindahl, H., Lundeheim, N., Einarsson, S., 2000. Plasma levels of cortisol, progesterone, oestradiol-17β and prostaglandin $F_{2\alpha}$ metabolite after ACTH (Synacthen® depot) administration in ovariectomized gilts. J. Vet. Med. A. 47, 193-200.

Pettersson, A., Einarsson, S., Kindahl, H., 1993. 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. Acta Vet. Scand. 34, 125-131.

Razdan, P., Mwansa, A.M., Kindahl, H., Rodriguez-Martinez, H., Hultén, F., Einarsson, S., 2002. Effect of repeated ACTH-stimulation on early embryonic development and hormonal profiles in sows. Anim. Reprod. Sci. 70, 127-137.

Rodriguez-Martinez, H., 2001. Oviduct function in cows and pigs: with special reference to sperm capacitation. Asian-Aust. J. Anim. Sci. 14, 28-37.

Rodriguez-Martinez, H., Einarsson, S., 1985. Influence of prostaglandins on the spontaneous motility of pig oviducts. Anim. Reprod. Sci. 8, 259-279.

Stookey, J.M., Gonyou, H.W., 1994. The effects of regrouping on behavioural and production parameters in finishing swine. J. Anim. Sci. 72, 2804-2811.

Sueldo, C.E., Oehninger, S., Subias, E., Mahony, M., Alexander, N.J., Burkr, L.J. Acosta, A.A., 1993. Effect of progesterone on human zona pellucida sperm binding and oocyte penetrating capacity. Fertil Steril. 60, 137-140.

Tsuma, V.T., Einarsson, S., Madej, A., Kindahl, H., Lundeheim, N., Rojkittikhun, T., 1996. Endocrine changes during group housing of primiparous sows in early pregnancy. Acta Vet. Scand. 37, 481-490.

Tsuma, V.T., Einarsson, S., Madej, A., Forsberg, M., Lundeheim, N., 1998. Plasma levels of cortisol and progesterone after ACTH administration in pregnant and lactating primiparous sows. Acta Vet. Scand. 39, 71-76.

Viring, S., 1980. Distribution of live and dead spermatozoa in the genital tract of gilts at different times after insemination. Acta Vet. Scand. 21, 587-597.

Viring, S., Einarsson, S., 1981. Sperm distribution within the genital tract of naturally inseminated gilts. Nord. Vet-Med. 33, 145-149.