Stress and Early Pregnancy in Sows

Effect on endocrinology, ova transport and embryo development

Pia Razdan

Department of Obstetrics and Gynaecology

Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2003

Acta Universitatis Agriculturae Sueciae
Veterinaria 153

ISSN 1401-6257
ISBN 91-576-6377-7

Tryck: SLU Service/Repro, Uppsala 2003

Till Max och Leo

GRISAR

Grisen, som är släkt med vildsvinet, började tämjas för 9000 år sedan. Det finns många olika grisar. De kan vara skära, fläckiga, bruna, svarta, håriga eller nakna. Grisar är allätare och tycker om att böka med trynet i jorden på jakt efter rötter, gräs och maskar. Ollon tycker de extra mycket om. Grisar är inga "lortgrisar". De rullar sig i leran för att svalka sig och för att insekter inte ska kunna sticka dem. Sin toalett har de på ett bestämt ställe. Pappan kallas gallt, mamman sugga och barnen kultingar. Suggorna kan få kultingar två gånger per år och ofta 10-12 stycken per gång, ibland flera. Kultingarna väljer en egen favoritspene och diar i 7-8 veckor om de får bestämma själva. Sedan kan de äta vanlig grismat. De växer mycket fort. Nyfödda kultingar väger drygt ett kilo. Ett halvt år senare väger de 100 kilo!

(Eriksson, I-K., *Tama djurungar*, 2001)

Abstract

Razdan, P. 2003. *Stress and early pregnancy in sows Effect on endocrinology, ova transport and embryo development*
Doctoral dissertation.
ISSN 1401-6257, ISBN 91-576-6377-7

Mixing unfamiliar breeding female pigs creates stressful situations due to aggressive interactions, which can lead to food deprivation of the more submissive animals. The aim of this thesis was to study the type of stress occurring when sows are regrouped into unfamiliar groups and to determine the effect of this type of stress during two different stages of pregnancy in multiparous sows.

Ova transport rate, embryo cleavage rate, the number of spermatozoa attached to the zona pellucida and the hormonal patterns were studied in sows subjected to food deprivation or ACTH-stimulation for 48 h, immediately after ovulation in their second oestrus after weaning. In sows subjected to the same treatments during the time of maternal recognition of pregnancy, the effect on hormonal concentrations in the blood plasma and allantoic fluid was examined, as well as the conceptus growth and foetal survival rate at day 30 of pregnancy.

Food deprivation decreased ova transport rate, which might be due to an impact of the elevated levels of $PGF_{2\alpha}$-metabolite on oviductal motility. Sows stimulated with ACTH had an increased level of cortisol and a decreased baseline level of $PGF_{2\alpha}$-metabolite. There was no difference in ova transport rate but ACTH-stimulated sows had decreased embryonic cleavage rate and a lower number of spermatozoa attached to the zona pellucida compared with controls, indicating an alteration in the oviductal environment.

Food deprivation and ACTH-stimulation during days 13 and 14 of pregnancy had no effect on embryo survival at day 30 of pregnancy, but both treatments increased the plasma levels of cortisol. ACTH-stimulation caused a two-day delay in the increase of plasma oestrone concentration seen at day 19 of pregnancy in control sows. This delay might reflect an impeded development of the embryos.

Plasma levels of cortisol, progesterone, and free fatty acids increased concomitantly with a decrease in insulin in food-deprived sows, indicating an adrenal activation as well as a catabolic metabolism with decreased metabolic clearance rate in the liver. Progesterone concentration in allantoic fluid among food-deprived sows was elevated. This increase in progesterone concentration was positively correlated with the weight of the placentas, suggesting an influence of progesterone on placenta size.

Post-ovulatory stress appears to alter the oviductal environment and post-ovulatory food deprivation also induces a decrease in ova transport rate. However, multiparous, well-nursed sows appear to have the capacity to compensate for moderate stress induced around the time of maternal recognition of pregnancy.

Keywords: ACTH, food deprivation, cortisol, progesterone, $PGF_{2\alpha}$-metabolite, oestrone, ova transport, cleavage rate and foetal development.

Author's address: Pia Razdan, Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Centre of Reproductive Biology, SLU, P.O. Box 7039, SE-750 07 Uppsala, Sweden.

Ett varmt tack till alla underbara suggor!

Contents

Appendix

List of original papers I-IV

The thesis is based on the following papers, which will be referred to in the text by Roman numerals:

I. Razdan, P., Mwanza, A.M, Kindahl H., Hultén, F., Einarsson, S. 2001. Impact of postovulatory food deprivation on the ova transport, hormonal profiles and metabolic changes in sows.
Acta veterinaria scandinavia 42, 15-25.

II. Razdan, P., Mwanza, A.M., Kindahl, H., Hultén, F., Einarsson, S. 2002. Effects of repeated ACTH-stimulation on early embryonic development and hormonal profiles in sows.
Animal Reproduction Science 70, 127-137.

III. Razdan, P., Tummaruk, P., Kindahl, H., Rodriguez-Martinez, H., Hultén, F., Einarsson, S. Hormonal profiles and embryo survival of sows subjected to induced stress during days 13 and 14 of pregnancy.
Manuscript submitted for publication.

IV. Razdan, P., Tummaruk, P., Kindahl, H., Rodriguez-Martinez, H., Hultén, F., Einarsson, S. The impact of stress during days 13 and 14 of pregnancy on the composition of allantoic fluid and conceptus development in sows.
Manuscript submitted for publication.

Abbreviations

ACTH	adrenocorticotropin
AIJ	ampullary-isthmic junction
bw	bodyweight
CL	corpora lutea
CRH	corticotropin-releasing hormone
GnRH	gonadotropin-releasing hormone
E_1SO_4	oestrone-sulphate
HPA	hypothalamic-pituitary-axis
LH	luteinizing hormone
PBS	phosphate buffered saline
PGE_2	prostaglandin E_2
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
POMC	pro-opiomelanocortin
POSPs	porcine oviductal secretory proteins
SA	sympathetic adreno-medullary
SD	standard deviation
UTJ	utero-tubal junction
VP	vasopressin
ZP	zona pellucida

Introduction

Background

Management procedures in modern intensive pig production include a number of practices that might act as stressors on the animals, e.g. high stocking densities, barren environments, transportation, poor or aggressive human-animal interactions (or neglect) and heat stress. In Sweden, group-housing systems for weaned sows are standard management practice in modern swine production on account of animal welfare considerations. Group housing of sows is considered to be less stressful for the animals because it allows them more opportunities to behave naturally. Housing systems where the movement of animals is restricted or prevented by, for example tethering, are known to cause chronic stress (Borell & Ladewig, 1989; Janssens, Helmond & Wiegant, 1995).

The drawback with the group-housing system is that regrouping of sows is unavoidable. In many group-housing systems, sows remain together after weaning for heat check and insemination. In some herds, sows are kept in intact groups until just before expected parturition when they are placed in separate pens. However, replacement gilts are introduced to the group during the gestation period. In other herds, the sows are regrouped after insemination in smaller groups where they are kept until they are placed in farrowing pens. There are many variants on the theme, but almost all of them include repeated regrouping of sows and the introduction of replacement gilts.

Pigs are social animals with a strong hierarchy within the group. Regrouping is therefore stressful for the animals. Regrouped sows require up to 48 h to establish a new rank-order. During this time, fights break out, which leads to increased cortisol levels and sometimes food deprivation of the more submissive animals (Mendl, Zanella & Broom, 1992; Brouns & Edwards, 1994; Tsuma *et al.*, 1996b). Since regrouping is often scheduled during the mating and early pregnancy period, it is important to evaluate the influence of stress on reproductive performance.

Stress

What is stress?

Stress can be defined in many ways but can be observed by the inability of an animal to cope with its environment, a phenomenon that is often reflected in a failure to perform according to the genetic potential (Dobson & Smith, 2000). Moberg (1993) describes stress as the biological response to an event that the individual perceives as a threat to its homeostasis. This event will be defined as a stressor. Perception of stressful stimuli leads to activation of the hypothalamic-pituitary-adrenal (HPA) axis, which in turn results in the release of a variety of peptides, principally corticotropin-releasing hormone (CRH) and vasopressin (VP; lysine vasopressin in pigs) from the hypothalamus (Buckingham, Cowell & Gillies, 1997). CRH stimulates the release of adrenocorticotropin (ACTH) and other pro-opiomelanocortin (POMC) derived peptides, such as β-endorphin from the anterior

lobe of the pituitary gland. ACTH acts on the adrenal cortex and causes secretion of glucocorticoid hormones, e.g. cortisol and corticosterone. Stress also involves the activation of the sympathetic nervous system and the adrenal medulla. This causes the release of catecholamines e.g. adrenaline and noradrenaline into the bloodstream, leading to an increase in the glucose supply by accelerating the degradation of glycogen in the liver (Vellucci, 1997a). The glucocorticoids also stimulate lipolysis and gluconeogenesis (the conversion of amino acids to glucose), which leads to an increased metabolism that promotes the ability to cope with stress.

Assessment of stress

Correct sampling procedures are fundamental for proper assessment of stress. It is essential that the techniques used do not impose further stress on the subject, which makes it clear that methods involving minimal handling and restraint as well as painless sampling have great advantages.

There are many difficulties involved in evaluating and comparing how different types of stress affect animal welfare in general, especially in a long-term stressful situation. Stress response can be assessed by determining the activation of the HPA-axis and/or the sympathetic adreno-medullary (SA) system, by measuring the level of secreted peptides in the peripheral blood plasma, urine, cerebrospinal fluid, saliva, etc. Behavioural responses such as heart rate, blood pressure and stereotypical behaviour, as well as the effects on the immune response can also be used for the assessment of stress response (for reviews see Kelley, 1988; Broom, 1991; Velluci, 1997b). However, meaningful evaluation of these responses requires a detailed knowledge of the normal physiological and behavioural patterns of the animal studied, because the response to stress is influenced by several factors such as the metabolic condition, health status, age and sexual maturity. The stress response is equally dependent on the nature, intensity and duration of the insult. Complicating factors include the large individual variation between pigs in their ability to cope with stress and the fact that each stressor has a non-specific (stimulation of the HPA-axis) and a specific effect. The latter is the "biological target" for the stressor. Heat stress, for example, leads to hyperthermia of the sow, food deprivation to a catabolic metabolism etc. For this reason, there is no stressor that can be used as a standard stressor to evaluate stress response in general. The specific response of each stressor has to be evaluated separately.

The duration of stress created by mixing unfamiliar animals will be 2 d at least, while the intensity will most likely depend on the size and constitution of the group. The nature of this type of stressor consists of a lack of control and fear (Broom, 1991), which will lead to an activation of the HPA-axis and finally an elevation of cortisol and catecholamine concentrations in peripheral blood plasma.

The influence of stress on reproductive functions

Reproduction is a basic requirement of all species and sows therefore make considerable physiological sacrifices to ensure good fertility. It is important to identify components of the reproductive process that are stress sensitive while easy to monitor.

In a reproducing female there is, in addition to the activation of the HPA- and SA-systems, also a cascade of other hormonal events that follows the immediate endocrine response to a stressor. These secondary events could lead to ovarian dysfunction, embryonic death and complete infertility (for review see Varley & Stedman, 1994). Although all reproduction may be vulnerable to stress, the weak points of the reproductive process are probably ovulation, expression of sexual behaviour and implantation of the embryo, because they are directly controlled by the neuroendocrine system. Most of the literature covering this subject is based on the effects of stress on the oestrous cycle and the release of luteinizing hormone (LH). Exposure to acute stressors might suppress gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus (Battaglia *et al.*, 1997), and stress via elevated levels of adrenal hormones may also decrease the pituitary responsiveness to GnRH (Moberg, 1985). This would result in a reduction in frequency and amplitude of the LH pulses secreted from the pituitary gland with subsequent abnormal ovarian function (Hennessy & Williamson, 1983; Dobson *et al.*, 1999; Dobson *et al.*, 2001).

The effect of stress on pregnancy seems to be very modest once implantation has occurred and the developing foetus is established (Moberg, 1985). Moreover, there are surprisingly few reports on the effects of stress on the more susceptible stage of pregnancy, e g. before and especially around the time of implantation.

The early pregnancy

Fertilisation, oviductal transport and embryo development

Mating occurs preferably before ovulation, which takes place approximately 24-48 h after the onset of oestrus, or at approximately two-thirds of the duration of oestrus (Soede, Noorhuizen & Kemp, 1992). During this relatively long pre-ovulatory oestrus, a sperm reservoir is established less than 30 min after mating, in the utero-tubal junction (UTJ), where spermatozoa are arrested and stored (Viring *et al.*, 1980; Hunter, 1981). The release and activation of spermatozoa at the time of ovulation is probably influenced by a local counter-current vascular transfer of ovarian follicular hormones (Hunter, 1983), which also influences the contractility of the isthmic muscles. The duration of ovulation is approximately 2 h in naturally ovulating loose-housed sows (Soede *et al.*, 1997). The newly ovulated ova reach the ampullary-isthmic junction (AIJ) where fertilisation takes place, within 45 min after ovulation. The ova remain at the AIJ for about 8-32 h and the number of spermatozoa attached to the zona pellucida (ZP) is, due to the controlled release from the UTJ, known to reflect the number of spermatozoa in the sperm reservoir and isthmus (Alanko, 1974). The total transport through the oviduct takes 48-56 h for both embryos and unfertilised ova (Mwanza *et al.*, 2002b). It is generally believed that the transport rate of ova is, among other factors, dependent on

oviductal motility (Rodriguez-Martinez *et al.*, 1982a; Mwanza, 2000), which is modified by circulating steroid hormones (Spilman, Shaikh & Harper, 1978).

Embryos are mainly in the 4-cell stage when entering the proximal part of the uterine horn, where they spend 2-3 d before being more evenly distributed throughout the uterus (Ashworth, 1991a). The pig blastocyst hatches from its glycoprotein covering, the ZP, 6-7 d after mating and begins to migrate within the uterine lumen until day 12 when it can no longer move freely. From day 12, pig blastocysts undergo a rapid 100-fold elongation, from an approximately 10-mm spherical stage to a 1 m-long filamentous form. At the same time, the conceptuses signal their presence by releasing oestrogens and possibly other factors that prohibit the regression of corpora lutea (CL) (Roberts, Sancai & Mathialagan, 1996). There is no true implantation, as seen in many other species, but attachment occurs during days 13 and 14. This process includes the establishment of a contact between trophoblasts and uterine membranes. Attachment is completed by about day 18 of gestation, when trophoblasts and uterine microvilli are fully interdigitated (Ashworth, 1991a). Placentation, defined as the differentiation of trophoblasts into the extra-embryonic membranes of the chorion, the amnion, yolk sac and allantois, occurs simultaneously with the elongation and attachment. The rapid elongation of the trophoblastic membrane (the placenta) is related to trophectodermal oestrogen production, which allows the conceptus to achieve a local stimulation of a large surface area of the endometrium (Geisert *et al.*, 1990). This local stimulation appears to be of great importance to prevent a return to oestrus, though it has been illustrated that at least two embryos in each uterine horn are required to maintain pregnancy (Polge, Rowson & Chang, 1966). Complete establishment of pregnancy requires a biphasic oestrogen surge.

As mentioned previously, concomitant with the rapid blastocyst elongation, an increased uterine content of oestrogens is seen on days 11-12 of pregnancy. Oestrogen levels decline on days 13-14, followed by a second sustained increase after day 14 (Geisert *et al.*, 1982; Stone & Seamark, 1985). Oestrogens produced by the blastocyst are believed to divert the endometrial and embryonic secretion of $PGF_{2\alpha}$ into the uterine lumen (exocrine) rather than towards the stroma (endocrine) and thereby protect the CL from $PGF_{2\alpha}$ exposure (Bazer & Thatcher, 1977). The exocrine diversion of $PGF_{2\alpha}$ is also expressed by the levels of $PGF_{2\alpha}$-metabolites in peripheral circulation. A complex series of $PGF_{2\alpha}$-metabolite peaks is observed during luteolysis but most of these peaks are not seen during early pregnancy (Shille *et al.*, 1979). These events, all essential to the maintenance of gestation, occur during a very short period of time, which makes this stage of pregnancy vulnerable to environmental influences.

Embryo mortality

In spontaneously ovulating pigs, managed under conditions considered to be optimal, fertilisation rates are high. Several studies have documented that embryo mortality in the pig is 20-40%, but large variations exist between and within pig populations (Lambert *et al.*, 1991). The majority of embryo mortality occurs before day 18 of pregnancy, but about 10% of the foetal loss occurs after day 40 when uterine crowding reduces litter size (Pope & First, 1985; Van der Lende, Soede &

Kemp, 1994). Parameters commonly used to assess viability of the embryo and quality of the microenvironment of the early embryo are conception rate, oviductal transport rate, cleavage and growth rate of the embryo, and the number of accessory spermatozoa attached to the ZP. Moreover, the within-litter diversity in embryo development has been correlated to increased embryo mortality (Pope, Maurer & Stormshak, 1982). Other parameters that might influence the quality of the embryo and its ability to survive are; blood plasma hormones associated with reproductive performance, such as progesterone, oestradiol-17β, oestrone, oestrone-sulphate (E_1SO_4), 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$-metabolite), as well as growth factors. The exact point at which sows are most sensitive to environmental influences during the early pregnancy is not fully evaluated.

Physiological factors such as breed differences, and non-physiological factors such as stress during early pregnancy affect the embryo mortality rate. One cause for embryo loss in sows is uterine asynchrony (Pope, 1988). Embryo mortality occurs when uterine secretions become altered in such a manner that development of the uterine environment is in asynchrony with the developing embryo. It is generally agreed that an abnormal change in blood plasma progesterone levels after ovulation might induce a slight asynchrony between embryo and uterine development, which in turn will reduce embryo viability (Pope, 1988). Mburu *et al.* (1998) found that in sows deprived of food immediately after ovulation, an increase in blood plasma progesterone was related to a retarded cleavage rate of the embryos and a decreased number of spermatozoa in the oviductal reservoir. The negative impact on embryo development seen in that study might have been caused by a progesterone-induced asynchrony between the embryos and the oviductal or uterine environment.

Prostaglandin is also suggested to be involved in the contractility of the oviduct (Petterson, Einarsson & Kindahl, 1993) and the timing of an increased level of prostaglandin seems to be of major importance for oviductal transport (Mwanza *et al.*, 2002a; 2002c). The concentration of prostaglandin $F_{2\alpha}$ in the oviductal fluid in the pig has a consistent relationship with the stage of the oestrous cycle (Rodriguez-Martinez, 1983). The highest concentration of prostaglandin $F_{2\alpha}$ in the oviductal fluid has been measured during standing heat at which time the highest oviductal motility *in vitro/vivo* has been reported to occur (Rodriguez-Martinez & Einarsson, 1985). It might therefore be speculated that there is a relationship between oviductal motility and the peripheral blood plasma level of prostaglandin. However, the mechanism by which post-ovulatory stress influences the endocrine status of the sow and thereby the synchrony between embryo development, ova transport and the maturation of the uterine environment is still to be investigated.

According to Hallford *et al.* (1975), $PGF_{2\alpha}$ is the endometrial luteolysin involved with regression of the CL after day 11 when the CL is no longer sensitive to $PGF_{2\alpha}$. At this point, any failure in the biphasic synthesis and release of conceptus oestrogens will lead to an abortion due to failure in redirecting endometrial $PGF_{2\alpha}$ into the uterine lumen. However, if there is large diversity in embryo development within litter at this stage of pregnancy, oestrogen production by the more advanced embryos will be detrimental to their less developed littermates.

Stress as a cause of reduced foetal survival

How and to what extent stress affects embryo survival is not evident. Heat stress has been reported to reduce implantation success and impair embryo development in sheep (Dutt, 1963). Gilts are more sensitive to heat stress before day 15 of pregnancy than during days 15-30 post-breeding (Edwards *et al.*, 1968). Omtvedt *et al.* (1971) illustrated a greater reduction in the number of viable embryos among gilts exposed to elevated temperatures during days 8-16 post-breeding than days 0-8, indicating that the time of implantation would be the stage of pregnancy most sensitive to heat stress. However, the activation of the HPA-axis is only one of several mechanisms involved when pigs are exposed to severely elevated temperatures.

Experiments aimed to evaluate the effect of elevated levels of adrenal hormones on early pregnancy have been performed by administration of ACTH. Arnold *et al.* (1982) observed a treatment × period interaction with the lowest percentage of foetal survival in gilts treated with ACTH on days 11-15 of gestation compared with the periods 1-5, 6-10 and 16-20 d of gestation. However, the embryo survival rate of control sows did not differ from the embryo survival rate in ACTH-administered sows. In this study, the plasma concentration of glucocorticoids was not measured, which makes it difficult to estimate the effects deriving from an activation of the HPA-axis. In later studies, ACTH administration was shown to increase blood plasma concentration of cortisol and progesterone in sows (Tsuma *et al.*, 1998; Mwanza *et al.*, 2000b) and gilts (Mwanza *et al.*, 2000c; Madej *et al.*, 2000).

Adrenal activation due to aggressive interactions occurs when pigs are mixed with unfamiliar individuals, but the effect of grouping on pregnancy is ambiguous. Bokma (1990) reported that sows placed in groups consisting of 25-30 animals during the first week after mating had 20% return to service and 10.5 piglets per litter compared with 10% and 10.7 respectively in sows grouped during the fourth week after mating. In contrast, gilts regrouped into groups consisting of 6 gilts around the time of mating had a higher overall pregnancy rate (86%) compared with gilts kept in tether-stalls (65%) during the same period (Barnett & Hemsworth, 1991). Low-ranked sows have higher plasma cortisol levels and a lower total weight of live-born piglets compared with more dominant sows (Mendl, Zanella & Broom, 1992; Tsuma *et al.*, 1996b). Consistent with these results, Bokma (1990) also reported that gilts group-housed with sows had lower litter size at birth compared with gilts grouped with gilts. From these studies, it might be concluded that the effect of group housing on the adrenal activation in an individual female depends on the rank-order of the individual itself, as well as the composition and size of the group. The effect of grouping on the profilicity of the female pig is also dependent on the stage of pregnancy at which the grouping is performed.

Regrouping of sows might lead to food deprivation of the more submissive animals (Brouns & Edwards, 1994). This results in an adrenal activation, observed in the form of elevated levels of cortisol and progesterone (Tsuma *et al.*, 1996a; Mburu *et al.*, 1998). Prolonged food deprivation of gilts leads to embryo loss,

increasing with the duration of starvation (Andersson, 1975). Food deprivation for 48 h after ovulation, which is a duration seen in regrouping situations, has been reported to have a negative effect on the cleavage rate of embryos and on the number of spermatozoa attached to the ZP. Food deprivation during days 10 and 11, on the other hand, did not cause a significant difference in embryo recovery rate at day 17 of pregnancy (Tsuma *et al.*, 1996a). There is evidence suggesting that nutrition, mediated by cortisol and insulin, may influence gonadotropin secretion through action at the hypothalamic-pituitary level. The ovarian response to gonadotropins might also be potentiated by increased plasma glucose, insulin and IGF-1 (Booth, Cosgrove & Foxcroft, 1996; Prunier & Quesnel, 2000), which would mean that a negative energy balance could directly lead to a decreased ovarian activity. However, the mechanism behind the effect of food deprivation on early pregnancy is not evident and needs to be further evaluated.

Aims

The overall objective of the present study was to mimic the stress occurring when sows are regrouped and to determine the effect of this type of stress during two different stages of pregnancy in multiparous sows. The specific aims within this context were to:

- Compare the effects of two different stressors imposed during the early pregnancy on embryo and foetal development and survival;
- describe how food deprivation immediately after ovulation in sows affects blood plasma hormonal concentrations and ova transport rate;
- determine the effect of repeated stimulation by synthetic ACTH during the first 48 h after ovulation on blood plasma hormone concentrations, embryo transport rate, embryo survival rate and number of spermatozoa attached to the ZP;
- study the effects of food deprivation and repeated simulation of ACTH for 48 h during days 13 and 14 of pregnancy on hormonal profiles in peripheral blood plasma;
- reveal the effects of the hormonal changes that follow stress in the form of food deprivation and repeated stimulation of ACTH for 48 h during the time of implantation on embryo survival rate, embryo growth and development and hormonal concentration in the allantoic fluid at day 30 of pregnancy in multiparous sows.

Methodological considerations

Materials and methods used in the present study are described in detail in the papers (I-IV) listed above, a more generalised description with special comments will be presented herein. The research plan and all procedures involving the use of animals were reviewed and approved by the Ethical Committee for Experimentation with Animals, Sweden.

Animals

In the present study, crossbred (Swedish Landrace × Swedish Yorkshire) sows were used: 21 in paper I, 22 in paper II and 17 each in papers III and IV (Table 1). All sows were brought from one commercial farm on the day of weaning to the Department of Obstetrics and Gynaecology, SLU, Uppsala, Sweden. To ensure proper detection of heat symptoms and stress-free access to feed, the sows were housed in individual pens (3m × 3m) on straw, in the vicinity of each other and a boar. To mimic the feeding routines in a commercial pig production unit as far as possible, the sows were fed 2.9 kg of a commercial ration according to Swedish standards (Simonsson, 1994). Water was provided *ad libitum*.

The experiments were carried out in the second oestrus after weaning to ensure that all sows were in an anabolic situation. From the day of arrival until the second oestrus after weaning, the sows were carefully monitored regarding their health status (appetite and general condition). In case of depression and/or inappetence, the body temperature was monitored and the sow was carefully examined. Sows that showed symptoms of illness and/or abnormal oestrus or oestrous interval were excluded from the study. All sows in the present study were randomly allocated to one out of three different groups.

Table 1. The number of sows in each experiment and treatment group

	Food-deprived	ACTH-stimulated	Controls
Paper I, no. of sows	11	—	10
Paper II, no. of sows	—	10	12
Paper III, no. of sows	5	6	6
Paper IV, no. of sows	5	6	6

Oestrous detection and ovulation

Heat detection was performed by using the back-pressure test in front of a boar. Standing oestrus was defined as when the sow responded with standing reflex to the back-pressure test in the presence of a boar. The first day of standing oestrus was defined as day 1 of the oestrous cycle/pregnancy.

The time of ovulation was determined through transrectal ultrasonography (Soede, Noorhuizen & Kemp, 1992; Mburu *et al.*, 1995) using a wagon especially constructed to immobilise sows during the time of scanning (Dalin *et al.*, 1995). The timing of ovulation in relation to oestrous symptoms in the first oestrus after

weaning was used to predict the interval from onset of standing oestrus to ovulation in the second oestrus after weaning for each sow (Mburu *et al.*, 1995). Ultrasonography was performed every fourth hour from about 20 h after the onset of oestrus until ovulation had occurred, and time of ovulation was set as the time when all large follicles had collapsed.

The sows were inseminated from 18-8 h prior to expected ovulation in the second oestrus after weaning (papers II-IV).

Treatments

In all sows, a jugular vein catheter was inserted under general anaesthesia (Rodriguez & Kunavongkrit, 1983) a few days before the second oestrus after weaning was expected. The anaesthesia was induced with an i.m. injection with azaperon (Stresnil® vet; 40 mg/ml) at a dose of 25 mg/kg bodyweight (bw) followed by a mixture composed of 5 ml romifidin and 500 mg tiletamin/zolazepam. The dose was 1.25 mg/kg bw of tiletamin/zolazepam (Zoletil® 100; 100 mg/ml), and 0.125 mg/kg bw of romifidin (Sedivet® vet; 10 mg/ml). The mixture was given as an i.m. injection about 10 to 15 min after the azaperon injection. The sows were kept on general anaesthesia by halothane inhalation gas.

In paper I, food deprivation started with the withdrawal of the first morning meal after ovulation was detected and continued until slaughter. In papers III & IV, sows were deprived of food from the morning of day 13 of pregnancy until the evening of day 14 and were provided with food in the morning of day 15. ACTH-stimulated sows (papers II-IV) were given intravenous injections of tetracosactid (Synachten® Depot), a synthetic ACTH, at a dose of 0.01 mg/kg bw every sixth hour. In paper II, the sows were treated from 4-8 h after ovulation until slaughter. In papers III & IV, ACTH stimulation started at 6 a.m. on day 13 and lasted until 6 a.m. on day 15 of pregnancy.

Blood sampling was performed every second hour during the treatment period, from approximately 12 h before ovulation until slaughter (papers I & II) and from day 13 until day 15 in paper III. In the latter paper, blood was also collected four times a day until the treatment period began on day 13 and from day 16 until slaughter.

Sows were slaughtered on average 43.3 h after ovulation in papers I & II. In papers III & IV, sows were slaughtered at 30±2 d of pregnancy. In all studies the genital tract was immediately recovered and examined and the number of CL, as well as the number of ova or foetuses, was counted.

Morphological examinations

The isthmic part of the oviduct was identified by palpation and this part was divided into three equally long segments (papers I & II). Each segment was flushed separately with phosphate buffered saline (PBS) as was the tip of the uterine horn.

The flushing fluid was examined under a light microscope and ova were counted. In paper II, recovered ova were placed in small glass petri dishes, examined with an inverted microscope with phase-contrast optics and classified as fertilised or non-fertilised. In fertilised ova, the cleavage rate and the number of accessory spermatozoa in the ZP were determined.

In paper IV, foetal survival rate was determined by dividing the total number of viable foetuses with the total number of CL. Foetuses were classified as viable or non-viable. The allantoic sacs were separated from each other and examined one-by-one, beginning with the sac situated nearest to the ovary in the left uterine horn. A sample of 10 ml of allantoic fluid was taken from each allantoic sac for hormonal analyses. The examination of each foetal unit included weight of total foetal unit, volume of allantoic fluid, weight and length of placenta, weight of allantochorion and weight and length of the foetus. Material for histological examination was taken from the foetal sacs located nearest to the ovaries and the uterine body, respectively. Histological examination was performed to determine differences regarding the area of erythropoietic cells in the liver of the foetuses and image-analysis was used for this purpose.

Hormone assays

Plasma cortisol was determined by radioimmunoassay (Coat-A-Count® Cortisol) (papers II & III). The main initial blood plasma metabolite of prostaglandin $F_{2\alpha}$ was analysed by radioimmunoassay as described previously by Granström & Kindahl (1982) and Kunavongkrit, Kindahl & Madej (1983) (papers I-III). The primary prostaglandins $F_{2\alpha}$ and E_2 were analysed in the amniotic fluid by a modified method of Lindgren, Kindahl & Hammarström, (1974). The concentration of plasma progesterone was determined using two different methods, an enzyme immunoassay (Amerlite) (papers I & II) and a solid-phase radioimmunoassay (Coat-A-Count® Progesterone) (paper III). Parallel samples were tested with the two methods, and the results were in full agreement (Å. Carlsson, Dept of Clinical Chemistry, SLU, Uppsala, Sweden, personal communication). A radioimmunoassay kit was used to analyse the concentration of oestrone and oestrone-sulphate in peripheral blood plasma (paper III). The concentration of insulin in peripheral blood plasma was analysed by radioimmunoassay according to Mburu *et al.* (1998) (paper I) and serum insulin was analysed using a solid-phase ^{125}I radioimmunoassay (Coat-A-Count® Insulin) (paper III). Blood serum glucose, free fatty acids (FFA) and triglycerides were analysed on Cobas FARA (papers I & III) using enzymatic methods (paper I). The concentration of progesterone, oestrone-sulphate, oestradiol-17β and prostaglandins in the allantoic fluid was determined using radioimmunoassays (paper IV).

Statistical analyses

Statistical evaluation was carried out using the SAS software package (SAS Institute Inc., 1989). To analyse differences between the treatment groups, the

Student's t-test in the MEANS procedure, Fisher´s Exact test (2-tail), analysis of variance in the GLM procedure and the MIXED procedure of the SAS software package were used. Data, which were not normally distributed, were transformed to logarithms and the repeated measurement analysis of variance was performed on the generated averages using the MIXED procedure. The CORR procedure, which performs Pearson's correlation analysis, was used to analyse possible associations between different variables. All statistical tests having a p-value of 0.05 or less were considered as significant.

Results

There were no differences between the treatment groups regarding the ovulation rate, ova recovery rate, number of foetuses, foetal survival rates and time of slaughter in any of the studies.

Morphological studies

Distribution of ova, cleavage rate of embryos, number of spermatozoa attached to the zona pellucida and development of the conceptuses

In paper I, food-deprived sows had 81.5% of their ova in the proximal and middle part of the isthmus while control sows had 78% of their ova in the distal part and in the uterus ($p<0.05$). In paper II, there were no differences between ACTH-stimulated sows and controls in the oviductal transport rate of the ova ($p=0.4$). The embryos of the ACTH-stimulated sows had a lower cleavage rate (2.4 cells/embryo) compared with the embryos of the control sows (2.8 cells/embryo) ($p=0.056$). Only 6 out of 10 ACTH-stimulated sows, while all 12 control sows, had embryos with more than 20 spermatozoa attached to the ZP ($p<0.05$). The average weight of the total foetal unit, the weight of foetuses, the crown-to-rump length, the weight of allantochorion and the volume of allantoic fluid were similar in control-, food deprived- and ACTH-stimulated sows and no differences in the diversity of size within litter could be found for any of these parameters (paper IV). The foetuses of the food-deprived sows had numerically heavier placentas (120 g) than both the control and the ACTH group (107 g and 103 g respectively) ($p=0.3$). The diversity in size of the placentas within litter was considerably lower in the control and food-deprived group (23 g and 22 g respectively) compared with the ACTH group (33 g) ($p=0.2$). The area of erythropoietic cells in the foetal livers did not differ between groups.

Patterns of hormones and blood serum metabolites

Cortisol

Due to an increase ($p<0.01$) in the level of cortisol following every ACTH stimulation, the average cortisol level at the time of treatment in ACTH-stimulated sows was 144±14 nmol/l (paper II) and 115±33 nmol/l (paper III). These average cortisol levels for ACTH-stimulated sows were significantly ($p<0.05$) higher than the average level of cortisol in control sows (63±15 and 62±36 nmol/l in papers II and III, respectively). Food-deprived sows had an elevated ($p=0.05$) cortisol level (110±62 nmol/l) on the first day of treatment (day 13) but during the second day (day 14), the cortisol level of the food-deprived sows was similar (61±31 nmol/l) to the level of the control sows (paper III). The food-deprived sows were also often restless and unsettled during the first day of treatment, but became more settled by the second day (paper III).

Progesterone

A gradual increase in the level of progesterone from the time of ovulation and onwards, which was not affected by treatment, was observed in all sows (papers I & II). Food deprivation during days 13 and 14 increased the level of progesterone to an average level of 107±6.9 nmol/l during days 13 to 15, which was significantly higher than the levels seen in the control group (75±5.5 nmol/l) and in the ACTH-stimulated sows (71±6.0 nmol/l) ($p<0.01$) (paper III). At day 30 of pregnancy, the progesterone concentration in the allantoic fluid was significantly higher in food-deprived sows (8.2±0.6 nmol/l) compared to control sows (6.6±0.5 nmol/l) and A-group sows (7.1±0.6 nmol/l) ($p<0.05$). Within the food deprived group, the concentration of progesterone in the allantoic fluid was positively correlated ($p<0.05$) with the placental size, which was not the case within the control and ACTH-stimulated group ($p=0.7$) at day 30 of pregnancy.

Oestrogens

In all sows, the plasma oestrone concentration was on average higher (27±6.6 pmol/l) during days 13 and 14 of pregnancy compared with the level during days 16 and 18 (9±8.9 pmol/l) (paper III). In the control and food-deprived group, a significant increase in the blood plasma level of oestrone starting at day 19 of pregnancy was observed, while the oestrone level in the ACTH group did not start to increase until day 21 of pregnancy. The oestrone level in ACTH sows was significantly ($p<0.05$) lower compared with the oestrone level of the control and food-deprived sows during days 19 to 22. There was no difference in the concentration of oestrone-sulphate in the blood plasma or in the allantoic fluid at day 30 of pregnancy, nor was there any difference between groups in the concentration of oestradiol-17β in the allantoic fluid at day 30 (paper III & IV).

Prostaglandins

In sows stimulated with ACTH directly after ovulation, the baseline level of $PGF_{2\alpha}$-metabolite decreased from about 300 pmol/l to 189±30 pmol/l from 8 h after the start of treatment and onwards ($p<0.05$) (paper II) (Fig.1). In sows deprived of food during the same period of time, the average level of $PGF_{2\alpha}$-metabolite was higher (546±42 pmol/l) compared with control sows (313±49 pmol/l) (paper I). An increase ($p<0.001$) in the blood plasma concentration of $PGF_{2\alpha}$-metabolite at day 12 of pregnancy was observed in all sows, irrespective of treatment. The first treatment of ACTH increased the level of $PGF_{2\alpha}$-metabolite (paper III) and subsequent injections gave a notable response in the level of $PGF_{2\alpha}$-metabolite but no significant increase. From 34-52 h after start of treatment, the level of $PGF_{2\alpha}$-metabolite was lower in the ACTH group compared with the other two groups. No difference ($p>0.05$) between groups in the concentrations of $PGF_{2\alpha}$-metabolite, $PGF_{2\alpha}$ and PGE_2 in the allantoic fluid at the time of slaughter was noted (paper IV).

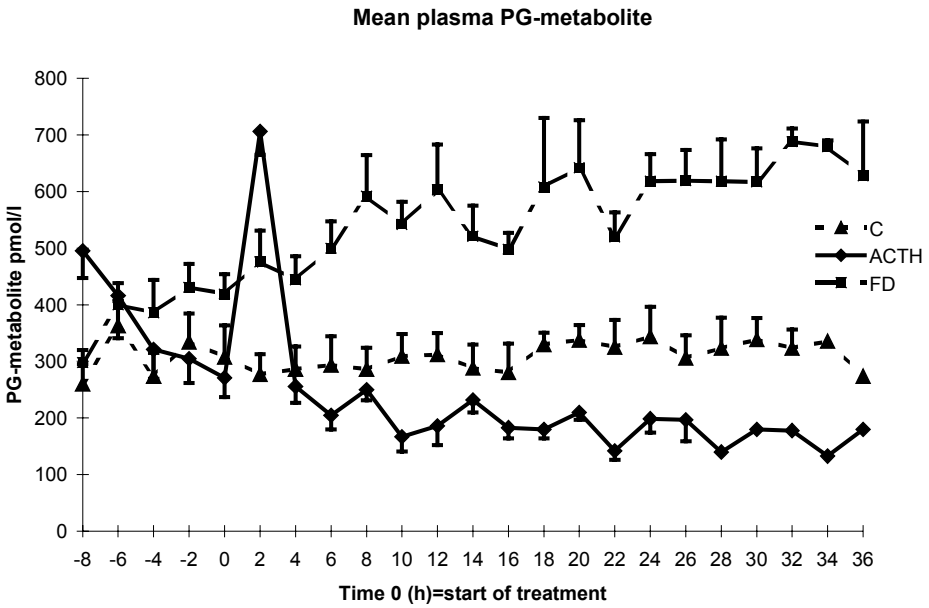

Figure 1. Plasma levels of $PGF_{2\alpha}$-metabolite (lsmeans±SD) directly after ovulation in control (C-), food-deprived (FD-) and ACTH-stimulated (ACTH) sows. The prostaglandin is depicted by a 2-h sampling frequency.

Insulin, free fatty acids, triglycerides and glucose

Food deprivation decreased ($p<0.001$) the concentration of insulin in blood plasma and increased ($p<0.01$) the level of free fatty acids but did not change the level of triglycerides and glucose compared with control sows. ACTH treatment, on the other hand, increased the levels of insulin ($p<0.001$) and triglycerides ($p<0.05$) but did not change the levels of free fatty acids compared with control sows (paper III).

General discussion

In the present study, the influence of stress on the reproductive functions was quite subtle. One reason for this might be that multiparous sows in their second oestrus after weaning were used. Sows, especially primiparous sows in the first oestrus after weaning, are more sensitive to stress because they are often in a catabolic state after weaning and around the first oestrus. Primiparous sows might also better reflect the situation in commercial piglet production. However, there were several experimental advantages with using multiparous sows in their second oestrus after weaning. The first oestrus after weaning was used to estimate time of ovulation in relation to the start of standing oestrus (Mburu *et al.*, 1995), to check that the sows had a normal oestrus and oestrous intervals, and that the sows were healthy. The obtained information was used to achieve an optimal timing of the different treatments during the experimental period. In the second oestrus, it could also be presumed that all sows had the same metabolic status, which was important in order to obtain an equal influence of food deprivation in all food-deprived sows. The advantage of sows compared with gilts is that sows are animals with a proven fertility. In addition, using gilts would have made the use of transrectal ultrasound examination difficult due to their narrower pelvis. Using a well-defined group of animals ensures a minimal difference in fertilisation rate between sows.

Sows in the present study were treated for 48 h because, it takes about 48 h to establish the new rank-order after regrouping (Tsuma *et al.*, 1996b). Forty-eight hours is also the approximate time that ova spend in the oviduct (Oxenreider & Day, 1965; Hunter, 1974; Mwanza *et al.*, 2002b). The sows were treated during two different stages of the oestrous cycle/pregnancy, both of which are believed to be critical for the outcome of pregnancy (Stroband & Van der Lende, 1990). The timing of embryonic entrance into the uterus is important (Chang, 1950), because the synchrony between the embryo development and the uterine environment might be disrupted if the time that the embryo spends in the oviduct is altered. During the time of maternal recognition, the maintenance of pregnancy is totally dependent on embryonic oestrogen production, although the production of other substances, such as proteins and prostaglandins, might be of importance. This stage of pregnancy might therefore be sensitive to alterations in the endocrine balance of the sow.

Oviductal transport time

The influence of oviductal milieu on embryo development

Food-deprived sows had a decreased oviductal transport rate compared with control sows, which resulted in each ovum spending a longer period of time in the oviduct and is consistent with the finding of Mwanza *et al*. (2000a). It has been suggested that embryo development is affected by ova transport rate and the composition of the oviductal fluid, where the latter is formed by selective transudation from the blood and active secretion from the endosalpinx (Hunter, 1988). The oviduct may secrete substances that affect cleavage rate (Fukui *et al.*, 1988), embryo viability (Gandolfi & Moore, 1987), the digestion of the ZP and also influence its function as a selective barrier (Broermann *et al.*, 1988). Mburu *et*

al. (1998) found a lower cleavage rate of embryos and a decreased number of spermatozoa attached to the ZP in sows deprived of food for 48 h. The delayed development might be due to an arrest in the oviduct, although it has been reported that embryos experimentally retained in the ampulla were able to develop to blastocysts but at slower rate (Pope & Day, 1972). Before hatching, the embryo development might be autonomous to some extent because most mammalian embryos (including pig embryos) have the potential to develop into the early blastocyst stage *in vitro* (Davis, 1985). There is evidence, however, that long-term culture leads to developmental disorders including reduced viability, delayed development and more variation within litter in the development, as well as a change in the metabolism of the embryos (Davis & Day, 1978; Nieman *et al.*, 1983; Papaioannou & Ebert, 1988).

The embryos of sows treated with ACTH directly after ovulation, on the other hand, were transported through the oviduct at the same speed as the embryos of the control sows, but still had a lower embryonic cleavage rate and fewer spermatozoa attached to the ZP. Previous studies indicate that biosynthetic activity of the oviduct is related to the hormonal status of the animal (for review see Buhi, Alvarez & Kouba, 1997). It can therefore be speculated that post-ovulatory stress due to hormonal changes influenced the oviductal milieu unfavourably in relation to embryo development. Oestrogen-dominated animals have been found to have greater biosynthetic activity than progesterone-dominated animals, but no difference in the biosynthetic activity was found between cyclic and early pregnant gilts (Buhi, Vallet & Bazer, 1989). Not only muscular and ciliary activity of the oviduct, but also the state of oedema at the UTJ and the longitudinal folds of isthmus, as well as the composition of the oviductal fluid regulate the number of spermatozoa in the oviductal reservoir (Hunter, 1981). The oviductal fluid contains specific tubal proteins, "porcine oviductal secretory proteins" (POSPs) that, among other things, might be related to the enhancement of spermatozoa-ZP binding (Buhi *et al.*, 1990; Buhi, Alvarez & Kouba, 1997). The treatment of the sows in the present study, started after the time of ovulation when the spermatozoa reservoir was already established in the lower part of isthmus and the controlled release of spermatozoa had begun (Hunter, 1984; Mburu *et al*., 1996). Lower number of spermatozoa attached to the ZP, as found at autopsy in the present study, could therefore indicate that the treatment had affected the release of spermatozoa from the reservoir, reflecting a disturbance of the oviductal milieu.

The role of the myosalpinx on gamete transport

The decreased ova transport rate in sows deprived of food could be due to endocrine changes either related directly to an altered metabolism or to changes associated to the stress response. These hormonal changes might have interfered with the oviductal milieu as well as affected oviductal motility. Exactly how the oviductal transport is regulated is still not fully understood as the ova are transported against the flow of tubal fluid. Nevertheless, it has been suggested that the adhesive interaction between cumulus cells and their extra-cellular matrix (principally hyaluronan), ciliary beating and myosalpingeal peristalsis are major components in the transport process (Tienthai *et al.*, 2000). Both the activity of the

autonomic nerve system and the endocrine status of the animal regulate the contractile pattern of the oviduct (Blandau *et al.*, 1977; Rodriguez-Martinez, 1983). The double layered myosalpinx is very richly innervated, with a high density of adrenergic nerve terminals permitting the isthmus to act as a physiological sphincter (Rodriguez-Martinez *et al.*, 1982b; Hunter, 1988). Rodriguez-Martinez (1984) illustrated *in vitro* how noradrenaline and adrenaline elicit both contractile (α-adrenergic) and relaxatory (β-adrenergic) isthmic responses, although a predominance of α-receptor-mediated effects was noticed in the isthmic circular layer and intact isthmus, especially during the post-ovulatory period. A high sensitivity was revealed in the pig isthmus *in vitro* to exogenous noradrenaline (Rodriguez-Martinez *et al.*, 1982b) and $PGF_{2\alpha}$ during the third day of the cycle, when ova are normally retained in the upper part of the isthmus. Rodriguez-Martinez & Einarsson (1982; 1985) suggested an interaction between the two groups of substances and the sphincter mechanism in the isthmic segment of the oviduct that would prevent premature entrance of the ova into the uterus.

Sows deprived of food immediately after ovulation in the present study had increased levels of $PGF_{2\alpha}$-metabolite while animals treated with ACTH during the same stage of oviductal transport had a decreased basal level of $PGF_{2\alpha}$-metabolite. Mwanza (2000) reported a decrease in the luminal isthmic pressure after ovulation, in control and ACTH-stimulated sows. This relaxation of the isthmic circular muscle appeared to be delayed in the food-deprived sows, and a high frequency of phasic pressure fluctuations in the isthmus during this period of time was noted instead. It is possible that the supposed stress-induced adrenergic response in the isthmic sphincter has to be enhanced by elevated $PGF_{2\alpha}$ concentrations to make a significant difference to the oviductal transport rate. These results are in agreement with the results of Mwanza *et al.* (2002a), who reported a decreased oviductal transport rate in sows after repeated administrations of $PGF_{2\alpha}$ in recently ovulated sows.

In sows treated with ACTH, the level of $PGF_{2\alpha}$-metabolite increased 1-2 h after administration, but the baseline level seemed to gradually decrease towards the end of the treatment period. This observation might be related to the ACTH-induced elevated level of cortisol, which has an inhibitory effect on the release of arachidonic acid. The peak in the level of $PGF_{2\alpha}$-metabolite seen after the ACTH stimulation is probably due to the fact that ACTH induces an enhanced conversion of ^{3}H-arachidonic acid to prostaglandins, as seen *in vitro* in cat adrenocortical cells (Laychock & Rubin, 1975). Moreover, these findings are consistent with earlier studies where ACTH-stimulation momentarily elevates $PGF_{2\alpha}$ synthesis in ovariectomized gilts (Mwanza *et al.*, 2000c) and castrated boars (Madej *et al.*, 2000). However, the fluctuations in the level of $PGF_{2\alpha}$-metabolite, with a constant decrease in the basal level, did not seem to have any effect on the oviductal transport rate in the present study. Contrary to food deprivation, ACTH stimulation has not been associated with any changes in oviductal activity (Mwanza *et al.*, 2000b), moreover decreased levels of $PGF_{2\alpha}$-metabolite have no effect on the ova transport time (Hultén *et al.*, 2000).

Endocrinology

Adrenal activation

In pigs, the peripheral concentration of cortisol is an accepted indicator of stress and also the predominant glucocorticoid hormone of the adrenal cortex (Heap, Holzbauer & Newport, 1966; Stott, 1981; Dantzer & Mormede, 1983). In the present study, the cortisol level of the sows in the two treatment groups was on average higher than the level seen after mixing of sows. However, the cortisol level in the two treatment groups did not remain elevated as long as it had been shown to do by Tsuma *et al.* (1996b) after regrouping in both dominant and subordinate sows. In sows deprived of food immediately after ovulation, the cortisol level was not measured because Mburu *et al.* (1998) and Tsuma *et al.* (1996a) have both reported that sows deprived of food had elevated cortisol levels throughout the treatment period, which corresponds to the treatment period in the present study. Sows deprived of food during days 13 and 14 in the present study had higher cortisol levels than control sows on the first day of treatment only, which indicates a quick adaptation to the situation. Blood samples collected every 20 min revealed that the cortisol concentration peaked approximately 1 h after the injection of tetracosactid and remained elevated for about 3-6 h, and the dose/response did not differ for doses between 0.005 and 0.015 mg/kg bw (Razdan, unpublished results). The dose/response was similar when treatment was done in the morning and in the afternoon even though physiological cortisol concentrations appear to be subject to a circadian rhythm, with higher morning than evening levels (Rafai & Fodor, 1980; Barnett, Cronin & Winfield, 1981; Janssens, Helmond & Wiegant, 1995). It must therefore be concluded that the blood sampling in the present study was frequent enough to detect the elevation of cortisol concentration that occurred due to adrenal activation by ACTH injections.

The levels of peripheral plasma adrenaline and noradrenaline were not measured in the present study. The release of catecholamines occurs very rapidly after stress exposure (Dalin *et al.*, 1993; Rozen, Tsuma & Magnusson, 1995) and the plasma half-life of the amines is extremely short (e.g. 70 s in rat plasma), which would have made it difficult to determine an accurate and yet comparable timing in the different treatments. Last but not least, the validity of measurement of venous blood plasma noradrenaline has been questioned, because regional or general differences in sympathetic activity may not be reflected accurately in venous blood samples taken from a single site (Vellucci, 1997b).

In the ACTH sows of the present study a peak in plasma progesterone was concomitant with a peak in cortisol levels immediately after ovulation. Since ACTH stimulation has been reported to increase the progesterone concentration in a similar fashion in ovariectomized ewes (Van Lier *et al.*, 1998) and gilts (Mwanza *et al.*, 2000c), the most likely source of progesterone in this case is the adrenal glands and not the ovaries. However, in the present study, the elevated progesterone concentration was only seen after the first ACTH administration and the reason for this is not clear. Progesterone is one of the steroid precursors for cortisol and it can be speculated that during persistent activation of the adrenal gland by ACTH, cortisol production would be favoured. Another explanation could be that the adrenal glands adapt to the ACTH stimulation or that the repeated

stimuli might change the responsiveness of the pituitary adrenocortical system to acute stressors; it could, however, be argued that there should be the same decrease in cortisol output. It is also possible that the progesterone peaks were not expressed as the level of progesterone, due to the increase of ovarian activity after ovulation. In the experiment carried out during days 13 and 14, an elevation in progesterone concentration was not detected due to infrequent blood sampling, although Tsuma *et al.* (1998) reported that plasma levels of progesterone were normalised within 120 min after ACTH injection in pregnant sows.

ACTH stimulation might also have enhanced the conversion of arachidonic acid into prostaglandin resulting in the rapid increase in $PGF_{2\alpha}$-metabolite seen after each administration. However, the elevated cortisol concentration appeared to inhibit the release of arachidonic acid, which led to a subsequent decrease in the basal level of $PGF_{2\alpha}$-metabolite.

Nutritional effects on endocrine status

One of several mechanisms that may contribute to nutritionally induced changes in the steroid hormone concentration in peripheral blood is the metabolic clearance rate of hepatic portal blood flow (Prime & Symonds, 1993; Adams *et al.*, 1994). A catabolic metabolism will lower the clearance rate, which might result in elevated $PGF_{2\alpha}$ -metabolite and progesterone levels. In sows deprived of food during the first 48 h after ovulation, no effect on progesterone concentration was noted, but there was a significant increase in the level of $PGF_{2\alpha}$-metabolite compared with controls. In sows deprived of food during days 13 and 14 of pregnancy the situation was quite the opposite; progesterone concentration was significantly elevated during the treatment period while there were no differences in the levels of $PGF_{2\alpha}$-metabolite between food-deprived and control sows. One explanation for this could be that there is a physiological rise in progesterone after ovulation, which could have concealed the effect of food deprivation on progesterone during this stage of pregnancy. Around days 12 to 14, on the other hand, the $PGF_{2\alpha}$-metabolite concentration increased dramatically in all sows regardless of treatment, which may have diminished the nutritional influence on the level of $PGF_{2\alpha}$-metabolite.

In the present study, food-deprived sows had a decreased insulin level and increased level of free fatty acids in comparison with control sows, reflecting a negative energy balance that they seemed to compensate for by catabolic metabolism and probably stress-induced lipolysis and glucogenesis. Many of the nutritional effects on embryo survival seem to be mediated by progesterone (for review see Foxcroft, 1997). However, reported effects of nutritionally induced changes in progesterone concentration on embryo development and survival are equivocal. Reduced plasma progesterone may mediate the detrimental effects of high-plane feeding on embryo survival in early pregnant gilts (Ashworth, 1991b; Jindahl *et al.*, 1996; Jindahl, Cosgrove & Foxcroft, 1997). On the other hand, induced elevation of progesterone concentration by feed restriction also has negative effects on embryo development (Mburu *et al.*, 1998) and survival (Tsuma *et al*., 1996a). Moreover, Mao & Foxcroft (1998) illustrated a negative correlation between progesterone supplementation during early pregnancy and embryo

survival. In the present study, the elevated progesterone concentration in blood plasma and allantoic fluid in the food-deprived sows compared with controls did not seem to have any effect on embryo survival at day 30 of pregnancy. However, the progesterone concentration in allantoic fluid of food-deprived sows was positively correlated with the placental size. Exogenous administration of progesterone and oestrone has been reported to elevate placental growth (Dalton & Knight, 1983). In addition, placenta size and relative efficiency are probably influenced by the level of oestrogens and possibly other uterine luminal growth factors at the time of elongation (Wilson & Ford, 2000). After day 30 of pregnancy, large placentas might induce intrauterine crowding, which can lead to reduced embryo survival rate (Tarraf & Knight, 1995). Before day 30, however, embryo survival is not affected by uterine space (Webel & Dziuk, 1974; Dziuk, 1985).

Embryo survival in sows treated during the time of maternal recognition of pregnancy.

In the present study, no differences in the embryo survival rate between food-deprived, ACTH-stimulated and control sows when slaughtered at day 30 of pregnancy were observed. The amount of oestrogens produced by the embryos is correlated with their stage of development. At the time of maternal recognition of pregnancy, the pig conceptus signals its presence by releasing oestrogens and other factors that prevent the regression of the CL (Roberts, Sancai & Mathialagan, 1996). In the present study, the blood plasma level of oestrone reflected the pattern of biphasic embryonic oestrogen production but the second sustained increase came 2 d later in the ACTH-stimulated sows compared with the control and food-deprived sows (day 21 and day 19, respectively). This could indicate an impeded development of the foetuses at this stage of pregnancy in ACTH-stimulated sows consistent with the impeded cleavage rate seen in embryos of sows stimulated with ACTH immediately after ovulation. However, there were no differences in growth rate and development of the embryos detected at the time of slaughter between ACTH and control sows.

Concluding remarks

For many reasons, regrouping in practice is likely to be more stressful for the female pig and more noxious for the embryo than the stress created in the present study. First of all, when animals are mixed in large groups, their cortisol level will probably be elevated for a longer period than 48 h, especially among submissive animals. In the study of Tsuma *et al.* (1996b), sows were placed in groups of only 3 animals and yet the cortisol level of the submissive animals was elevated for no less than 3 d. In practice, stress caused by food deprivation or food restriction will be added to social stress and not be a separate feature as in the experimental model of the present study. Moreover, the animals in commercial herds are not likely to be as a homogenous group as the multiparous sows used in this study. Gilts and primiparous sows will often belong to the more submissive animals and they seem to be more sensitive to hormonal changes as a result of stress and nutritional

changes. In a commercial herd, sows will also be bred in their first oestrus after weaning.

The purpose of the present study was to investigate and estimate the effects of stress that unfamiliar, breeding female pigs might be exposed to during a grouping situation using as controlled experimental conditions as possible. The stressors used—food deprivation and ACTH stimulation—had significant effects on the endocrine status of the sows during the treatment period, both when treatments were performed immediately after ovulation and during days 13 and 14 of pregnancy. Post-ovulatory stress in the form of ACTH stimulation and food deprivation delayed the embryonic cleavage rate and decreased the number of spermatozoa attached to the ZP, reflecting a change in the oviductal milieu. Post-ovulatory food deprivation also impeded the oviductal transport rate, which may be due to a prostaglandin-mediated prolonged contraction of the isthmic muscle. When the treatments were performed during days 13 and 14 however, there were no significant effects on embryo growth rate and survival observed at day 30 of pregnancy. The progesterone concentration of the allantoic fluid of food-deprived sows at day 30 was, however, increased compared with controls and also correlated to the size of the placentas. The absence of treatment effects on the embryo survival at day 30 might be due to: 1. Large individual variation in the ability to cope with stress in combination with relatively small experimental groups; 2. the degree of stress employed was not severe enough, or the sows were not exposed to the treatments for a long enough period of time. It must also be taken into account that the sows used in the study were in very good nutritional and physical condition and as reproduction is the most important activity of any species, the capacity to compensate for the effects of stress is very well developed (Dobson *et al.*, 2001).

The results of the present study suggest that the impact of stress on embryo development is greater the earlier the pregnant sow is exposed to stressors. This could be due to an ability of the sows to compensate for the effects of stress or that sensitivity of the embryos to disturbances in the maternal endocrinology decreases with time.

Future prospects

Concentrations of hormones and metabolites in the peripheral circulation, which was assessed in the present study, are not always correlated with concentrations occurring in the local counter-current system working between the utero-ovarian vein and artery. The counter-current system creates concentrations of different hormones specific for that region, and changes in these regional levels influence the oviductal motility and environment at the time of ovulation and oviductal transport (Pharazyn, Foxcroft & Aherne, 1991). Therefore, it would be of much interest to measure the local steroid concentrations in the utero-ovarian vein in animals exposed to stress during this period of gestation. Further knowledge would also be added by analysis of steroid receptors and mRNA expression in the oviductal tissue.

The impact of stress before and during the time of fertilisation on oocyte quality and cleavage rate of the fertilised ova, as well as the oviductal transport rate merits further investigation. Stress, during this period might have a great influence on the oviductal milieu and motility and secondly on embryo development. It would also be of great interest to measure the plasma levels of hormones and cathecolamines in the utero-ovarian vein and the oviductal fluid, as well as the adrenergic receptor expression in the oviductal tissue. In many production units, the sows are regrouped at the day of weaning and the effects of stress before and during mating are therefore of great interest.

Grouping of sows might lead to food deprivation of the more submissive sows. In a real-life situation, the low-ranked sows are exposed to adrenal activation due to fear and loss of control at the same time as they might be deprived of food. This situation lasts for approximately 48 h, but low-ranked sows might have elevated cortisol levels for a longer period of time (Tsuma *et al.,* 1996a). To mimic the real-life situation for a low-ranked sow in a better way, the period of ACTH stimulation could be prolonged to 72 h and the sows should also be deprived of food during the first 48 h. In addition, it would be interesting to compare the results from sows with results gained from gilts, primiparous sows, or sows in their first oestrus after weaning with a known catabolic metabolism, after undergoing the same treatment.

Conclusions

- Food deprivation induced a decrease in ova transport rate, which appeared to be mediated by elevated plasma levels of $PGF_{2\alpha}$.

- The hormonal changes seen after ACTH stimulation might have altered the oviductal milieu and thereby caused the decrease seen in embryonic cleavage rate and number of spermatozoa attached to the ZP, although there was no effect of ACTH stimulation on the ova transport rate.

- Plasma levels of cortisol, progesterone and free fatty acids increased whereas insulin levels decreased in food-deprived sows, indicating an adrenal activation, as well as a catabolic metabolism that may have caused a decreased metabolic clearance rate in the liver.

- ACTH stimulation caused an adrenal activation, reflected by the increased levels of cortisol and progesterone. ACTH stimulation might also have enhanced the conversion of arachidonic acid into prostaglandin resulting in the rapid increase in $PGF_{2\alpha}$-metabolite seen after each administration.

- ACTH stimulation during days 13 and 14 caused a 2-d delay in the increase of plasma oestrone concentration, which occurred at day 19 of pregnancy in control sows. This might reflect an impeded development of the embryos.

- The allantoic fluid of food-deprived sows had an increased progesterone concentration, which was positively correlated with the weight of the placentas, suggesting that progesterone influenced placenta size among food-deprived animals.

- Multiparous, well-nursed sows seem to have the capacity to compensate for the influence of moderate stress induced around the time of maternal recognition of pregnancy.

References

Adams, N.R., Abordi, J.A., Briegel, J.R., Sanders, M.R. 1994. Effect of diet on the clearance of estradiol-17β in the ewe. *Biology of Reproduction 51,* 668-674.

Alanko, M., 1974. *Fertilization and early development of ova in AI-gilts, with special reference to role of tubal sperm concentration: A clinical and experimental study.* Ph.D. Thesis, Helsinki .

Andersson, L.L. 1975. Embryonic and placental development during prolonged inanition in the pig. *American Journal of Physiology 229*, 1687-1694.

Arnold, J.D., Kattesh, H.G., Chen, T.T., Murphree, R.L. 1982. Adrenocorticotrophin administration during early gestation on conceptus development in swine. *Theriogenology, 17,* 475-484.

Ashworth, C.J. 1991a. Embryo development. *Pigs News and Information* 12, 551-554.

Ashworth, C.J. 1991b. Effect of pre-mating nutritional status and post-mating progesterone supplementation on embryo survival and conceptus growth in gilts. *Animal Reproduction Science 26,* 311-321.

Barnett, J.L., Cronin, G.M., Winfield, C.G. 1981. The effects of indicidual and group penning of pigs on total and free plasma corticosteroids and the maximum cortiscosteroid binding capacity. *General and Comparative Endocrinology 44,* 219-225.

Barnett, J.L., Hemsworth, P.H. 1991. The effects of individual and group housing on sexual behaviour and pregnancy in pigs. *Animal Reproduction Science 25,* 265-273.

Battaglia, D.F., Bowen, J.M., Krasa, H.B., Thrun, L.A., Viguié, C., Karsch, F.J. 1997. Endotoxin inhibits the reproductive neuroendocrine axis while stimulating adrenal steroids: A simultaneous view from hypophyseal portal and peripheral blood. *Endocrinology 138,* 4273-4281.

Bazer, F.W., Thatcher, W.W. 1977. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin $F_{2\alpha}$ by the uterine endometrium. *Prostaglandins 14,* 397-401.

Blandau, R.J., Brackett, B., Brenner, R.M., Boling, J., Broderson, S., Hammer, C., Mastroianni, L. 1977. The Oviduct. In: *Frontiers in reproduction and fertility control. A review of the reproductive sciences and contraceptive development.* R.O. Greep and M.A. Koblinsky, MIT Press. Cambridge, UK. Part II, 132-145

Bokma, S., 1990. Housing and management in practise: Partly slatted systems. In: *Electronic Identification in Pig Production.* Monogr. Ser. No. 10, Royal Agricultural Society, UK, 37-46.

Booth, P.J., Cosgrove, J.R., Foxcroft, G.R. 1996. Endocrine and metabolic responses to realimentation in feed-restricted prepubertal gilts: Associations among gonadotropins, metabolic hormones, glucose and uteroovarian development. *Journal of Animal Science 74*, 840-848.

Borell von, E., Ladewig, L. 1989. Altered adrenocortical response to acute stressors or ACTG(1-24) in intensively housed pigs. *Domestic Animal Endocrinology, 6,* 299-309.

Broermann, D.M., Xie, S., Nephew, K.P., Pope, W.F. 1988. Ability of the oviduct to delay enzymatic digestion of the zona pelucida. *Journal of Animal Science Suppementl 1,* 66, 216 abstr.

Broom, D.M. 1991. Animal Welfare: Concepts and measurement. *Journal of Animal Science 69,* 4167-4175.

Brouns, F., Edwards, S.A. 1994. Social rank and feeding behaviour of group-housed sows fed competitively or ad libitum. *Applied Animal Behaviour Science 39,* 225-235.

Buckingham, J.C., Cowell, A-M., Gillies, G.E. 1997. The neuroendocrine system: Anatomy, physiology and responses to stress. *In: Stress, Stress Hormones and the Immune System.* Edited by John Wiley & Sons, Ltd., 9-47.

Buhi, W.C., Alvarez, I.M., Kouba, A.J. 1997. Oviductal regulation of ferilization and early embryonic development. *Journal of Reproduction & Fertility Supplement 52,* 285-300.

Buhi, W.C., Alvarez, I.M., Sudhipong, V., Dones-Smith, M.M. 1990. Identification and characterisation of de novo-synthesized porcine oviductal secretory proteins. *Biology of Reproduction 43,* 929-938.

Buhi, W.C., Vallet, J.L., Bazer, F.W. 1989. De novo synthesis and release of polypeptides from cyclic and early pregnant procine oviduct al tissue in explant culture. *The Journal of Experimental Zoology 252,* 79-88.

Chang, M.C. 1950. Development and fate of transferred rabbit ova or blastocysts in relation to the ovulation time of recipients*, Journal of experimental Zoology 144,* 197-225.

Dalin, A-M., Magnusson, U., Häggendal, J., Nyberg, L. 1993. The effect of transport stress on plasma levels of catecholamines, cortisol, corticosteroid-binding globulin, blood cell count and lymphocyte proliferation in pigs. *Acta veterinaria scandinavica 34,* 59-68.

Dalin, A-M., T. Nanda, Hultén, F., Einarsson, S. 1995. Ovarian activity at naturally attained oestrus in the sow, an ultrasonographic and LH study. *Acta veterinaria scandinavia 36,* 377-382.

Dalton, D.L., Knight, J.W. 1983. Effects of exogenous progesterone and estrone on conceptus development in swine. *Journal of Animal Science 56,* 1354-1361.

Dantzer, R., Mormede, P.1983. Stress in farm animals: A need for re-evaluation. *Journal of Animal Science 57,* 6-18.

Davis, D.L. 1985. Culture and storage of pig embryos. *Journal of Reproduction & Fertility Supplement 33,* 115-124.

Davis, D.L., Day, B.N. 1978. Cleavage and blastocyst formation by pig eggs in vitro. *Journal of Animal Science 46,* 1043-1053.

Dobson, H., Campbell, B.K., Scaramuzzi, R.J., Blaird, D.T. 1999. Effect of reducing LH pulse frequency and amplitude on ovarian oestradiol production in the ewe. *5th International Symposium on Reproduction in Domestic Ruminants. Journal of Reproduction & Fertility Supplement 54,* 510.

Dobson, H., Smith, R.F. 2000. What is stress, and how does it affect reproduction? *Animal Reproduction Science 60-61,* 743-752.

Dobson, H., Tebble, J.E., Smith, R.F., Ward, W.R. 2001. Is stress really all that important? *Theriogenology 55*, 65-73

Dutt, R.H. 1963. Critical period for early embryo mortality: ewes exposed to high ambient temperature. *Journal Animal Science 22,* 713-725.

Dziuk, P.J. 1985. Effect of migration, distribution and spacing of pig embryos on pregnancy and fetal survival. *Journal of Reproduction & Fertility Supplement 33*, 57-63.

Edwards, R.L., Omtvedt, I.T., Turman, E.J., Stephens, D.F., Mahoney, G.W.A. 1968. Reproductive performance of gilts following heat stress prior to breeding and in early gestation. *Journal Animal Science 27*, 1634-1637.

Foxcroft, G.R. 1997. Mechanisms mediating nutritional effects on embryonic survival in pigs. *Journal of Reproduction & Fertility Supplement 52,* 47-61.

Fukui, Y., Glew, A.M., Gandolfi, F., Moore, R.M. 1988. In vitro culture of sheep oocyte matured and fertilized in vitro. *Theriogenology 29,* 883-391.

Gandolfi, F., Moore, R.M. 1987. Stimulation of early embryonic development in the sheep by coculture with oviduct epithelial cells. *Journal of Reproduction & Feritlity 81,* 23-28

Geisert. R.D., Renegar, R,H., Thatcher, W.W., Roberts, R.M., Bazer, F.W. 1982. Establishment of pregnancy in the pig: Interrelationships between pre-implantation development of the pig blastocyst and uterine endometrial secretions. *Biology of Reproduction 27,* 925-939.

Geisert, R.D., Zavy, M.T., Moffatt, R.J., Blair, R. M., Yellin, T. 1990. Embryonic steroids and the establishment of pregngncy in pigs. *Journal of Reproduction & Fertility Supplement 40,* 293-305.

Granström, E., Kindahl, H. 1982. Radioimmunoassay of the Major Plasma Metabolite of $PGF_{2\alpha}$ 15-Keto-13,14-dihydro-$PGF_{2\alpha}$, *Metods in Enzymology.* Academic Press Inc., 86, 320-339.

Hallford, D.M., Wetterman, R.P., Turman, E.J., Omtvedt, I.T. 1975. Luteal function in gilts after prostaglandin $F_{2\alpha}$. *Journal of Animal Science 41,* 1706-1710.

Heap, R.B., Holzbauer, M., Newport, H.M. 1983. Adrenal secretion rates of C-19 and C-21 steroids before and after hypophysectomy in the pig and the dog. *Journal of Endocrinology 36,* 159.

Hennessy, D.P., Williamson, P. 1983. The effect of stress and of ACTH administration in hormone profiles, oestrous and ovulation in pigs. *Theriogenology 20,* 13-26.

Hultén, F., Tanasuparuk, W., Englund, P., Kindahl, H., Einarsson, S. 2000. The influence of inhibited prostaglandin biosynthesis on post-ovulatory oviductal transport in sows. *Theriogenology, 53,* 1397-1405.

Hunter, R.H.F. 1974. Chronological and cytological details of fertilization and early embryonic development in the domestic pig, Sus scrofa. *Anatomical Records 178,* 169-186.

Hunter, R.H.F. 1981. Sperm transportation and reservoirs in the pig oviduct in relation to the time of ovulation. *Journal of Reproduction & Fertility 63,* 109-117.

Hunter, R.H.F. 1983. Regulation of oviduct function in pigs by local transfer of ovarian steroids and prostaglandins: a mechanism to influence sperm transport. *European Journal of Obstetric and Gynaecology Reproductive Biology 14,* 225-232

Hunter, R.H.F. 1984. Pre-ovulatory arrest and peri-ovulatory redistribution of competent spermatozoa in the isthmus of the pig oviduct. *Journal of Reproduction & Fertility 72,* 203-211.

Hunter, R.H.F. 1988. Their role in fertility and infertility. *The Fallopian tube*. Springer-Verlag, Berlin, Heidelberg, 22-126.

Janssens, C.J.J.G., Helmond, F.A., Wiegant, V.M. 1995. The effect of chronic stress on plasma cortisol concentrations in cyclic female pigs depends on the time of the day. *Domestic Animal Endocrinology 12*, 167-177.

Jindal, R., Cosgrove, J.R., Aherne, F.X., Foxcroft, G. R. 1996. Effect of nutrition on embryonal mortality in gilts: Association with progesterone. *Journal of Animal Science 74,* 620-624.

Jindal, R., Cosgrove, J.R., Foxcroft, G. R. 1997. Progesterone mediates nutritionally induced effects on embryonic survival in gilts. *Journal of Animal Science 75,* 1063-1070.

Kelley, K.W. 1988. Cross-talk between the immune and endocrine systems. 1988. *Journal of Animal Science 66,* 2095-2108.

Kunavongkrit, A., Kindahl, H., Madej, A. 1983. Clinical and endocrinological studies in primiparous zero-weaned sows. Hormonal patterns of normal cycling sows after zero-weaning. *Zentralblatt für Veterinärmedizin 30,* 616-624.

Lambert, E., Williams, D.H., Lynch, P.B., Hanarahan, T.J., McGeady, T.A., Austin, F.H., Boland, M.P., Roche, J.F. 1991. The extent and timing of prenatal loss in gilts. *Theriogenology 36,* 655-665.

Laychock, S.G., Rubin, R.P. 1975. ACTH-induced prostaglandin biosynthesis from 3H-arachidonic acid by adreocortical cells. *Prostaglandins 10*, 529-540.

Lindgren, J.Å., Kindahl, H. and Hammarström, S. 1974. Radioimmunoassay of prostaglandins E_2 and $F_{2\alpha}$ in cell culture media utilizing antisera against prostaglandins $F2_{2\beta}$ and $F2_{2\alpha}$. *FEBS Letter 48,* 22-25.

Madej, A., Mwanza, A,M., Kindahl, H., Einarsson, S. 2000. The effect of ACTH and CRH on plasma levels of cortisol and prostaglandin $F_{2\alpha}$ in cycling gilts and castrated boars. *Proceedings of the 14th International Congress on Animal Reproduction,* Stockholm, Sweden 2, 61.

Mao, J., Foxcroft, G.R. 1998. Progesterone therapy during early pregnancy and embryonal survival in primiparous weaned sows. *Journal of Animal Science 76*, 1922-1928.

Mburu, J.N., Einarsson, S., Dalin, A-M., Rodriguez-Martinez, H. 1995. Ovulation as determined by transrectal ultrasonography in multiparous sows: relationship with oestrous symptoms and hormonal profiles. *Journal of Veterinary Medicine, 42,* 285-292.

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. *Animal Reproduction Science 45,* 109-121.

Mburu, J.N., Einarsson, S., Kindahl, H., Madej, A., Rodriguez-Martinez, H. 1998. Effect of post-ovulatory food deprivation on oviductal sperm concentration, embryo development and hormonal profiles in the pig. *Animal Reproduction Science*,*52,* 221-234.

Mendl, M., Zanella, A.J., Broom, D.M. 1992 Physiological and reproductive correlates of behavioural strategies in female domestic pigs. *Animal Behaviour 44,* 1107-1121.

Moberg, G.P. 1985. Influence of stress on reproduction: Measure of well-being. *Animal Stress* American Physiological Society, Maryland, US, 245-267.

Moberg, G.P. 1993. A review-Developing management strategies to reduce stress in pigs: A new approach utilizing the biological cost of stress. *Manipulating Pig Production IV, Australiaasian Pig Science Association*, Attwood, ES Batterham. 116-126.

Mwanza, A.M. 2000. *Oviductal isthmic motility and the effect of stress thereupon and on the endocrine status and ova transport of recently ovulated pigs.* Ph.D. Thesis, ISBN 91-576-5930-3, SLU, Uppsala. 32 pp.

Mwanza, A.M., Einarsson, S., Madej, A., Lundeheim, N., Rodriguez-Martinez, H., Kindahl, H. 2002a. Postovulatory effect of repeated administration of prostaglandin $F_{2\alpha}$ on the endocrine status, ova transport, binding of accessory spermatozoa to the zona pellucida and embryo development of recently ovulated sows. *Theriogenology 58,* 1111-1124.

Mwanza, A.M., Englund, P., Kindahl, H., Lundeheim, N., Einarsson, S. 2000a. Effects of postovulatory food deprivation on the hormonal profiles, activity of the oviduct and ova transport in sows. *Animal Reproduction Science 59,* 185-199.

Mwanza A.M., Madej, A., Kindahl, H., Lundeheim, N., Einarsson, S. 2000b. Postovulatory effect of repeated intravenous administration of ACTH on the contractile activity of the oviduct, ova transport and endocrine status of recently ovulated and unrestrained sows. *Theriogenology 54,* 1305-1316.

Mwanza, A.M., Madej, A., Kindahl, H., Lundeheim, N., Einarsson, S. 2000c. Plasma levels of cortisol, progesterone, oestradiol-17β, and prostaglandin F2α metabolite after ACTH (Synacthen Depot®) administration in ovariectomized gilts. *Journal of Veterinary Medicine 47*, 193-200.

Mwanza, A.M., Razdan, P., Hultén, F., Einarsson, S. 2002b. Transport of fertilised and unfertilized ova in sows. *Animal Reproduction Science 74*, 69-7

Mwanza, A.M., Rodriguez-Martinez, H., Kindahl, H., Einarsson, S. 2002c. Postovulatory effect of intravenous administration of lipopolysaccharide (E. coli, O55:B5) on the contractility activity of the oviduct, ova transport, binding of accessory spermatozoa to the zona pellucida and embryo development in sows. *Reproduction of Domestic Animal 37,* 269-274.

Nieman, H., Illera, M.J., Dziuk, P.J. 1983. Developmental capacity, size and number of nuclei in pig embryos cultured in vitro. *Animal Reproduction Science 5,* 311-322.

Omtvedt, I.T., Nelson, R.E., Edwards, R.L., Stephens, D.F., Turman, E.J. 1971. Influence of heat stress during early, mid and late pregnancy of gilts. *Journal of Animal Science 32,* 312-317.

Oxenreider, S.L., Day, B.N. 1965. Transport and cleavage of ova in swine. *Journal of Animal Science 24,* 413-417.

Papaioannou, V.E., Ebert, K.M. 1988. The preimplantation pig embryo: cell number and allocation to trophectoderm and inner cell mass of the blastocyst in vivo and in vitro. *Development 102,* 793-803.

Petterson, 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 intra-luminal pressure variations in the isthmus of the oviduct in unrestrained gilts. *Acta veterinaria scandinavia 34,* 125-131.

Pharazyn, A., Foxcroft G.R., & Aherne, F.X. 1991. Temporal relationship between plasma progesterone concentration in the utero-ovarian and jugular veins during early pregnancy in the pig. *Animal Reproduction Science 26,* 323-332.

Polge, C., Rowson, L.E.A., Chang, M.C. 1966. The effect of reducing the number of embryos during early stages of gestation on the maintenance of pregnancy in the pig. *Journal of Reproduction & Fertility 12,* 395-397.

Pope, W.F. 1988. Uterine asynchrony: A cause of embryonic loss *Biology of Reproduction 39,* 999-1003

Pope, W.F., Day, B.N. 1972. Development if pig embryos following restriction to the ampullar portion of the oviduct. *Journal of Reproduction & Fertility 31,* 135-138.

Pope, W.F., First, N.L. 1985. Factors affecting the survival of pig embryos. *Theriogenology 23,* 91-105

Pope, W.F., Maurer, R.R., Stormshak, F. 1982. *Proceedings of the Society for Experimental Biology and Medicine 171,* 179-183

Prime, G.R., Symonds, H.W. 1993. The influence of plane of nutrition on portal blood flow. *Journal of Agricultural Science 121,* 389-397.
Roberts, M. R., Sancai, X., Mathialagan, N. 1996. Maternal Recognition of Pregnancy. *Biology of Reproduction 54,* 294-302.
Prunier, A., Quesnel, H. 2000. Nutritional influences on the hormonal control of reproduction in female pigs. *Livestock Production Science 63*, 1-16
Rafai, P., Fodor, È. 1980. Studies on porcine adrenaocortical function. *Acta veterinaria academiae scientiarum Hungaricae 28,* 433.441.
Roberts, M. R., Sancai, X., Mathialagan, N. 1996. Maternal Recognition of Pregnancy. *Biology of Reproduction 54,* 294-302.
Rodriguez-Martinez, H. 1983. *Studies on the control mechanisms of the pig oviductal motility.* Ph.D. Thesis, ISBN 91-576-1621-3, SLU, Uppsala, 24-25.
Rodriguez-Martinez, H. 1984. Effects of adrenergic agents on the in vitro motility of porcine oviducts. *Zbl Vet Med. A 31,* 91-104.
Rodriguez-Martinez, H., Einarsson, S. 1982. Spontaneous contractility of isolated muscle layers of the pig ovuductal isthmus. *Acta Physiologica Scandinavica 115,* 325-330.
Rodriguez-Martinez, H., Einarsson, S. 1985. Influence of prostaglandins on the spontaneous motility of pig oviducts. *Animal Reproduction Science 8,* 259-279.
Rodriguez-Martinez, H., Einarsson, S., Larsson, B., Akusu, M., Settergren, I. 1982a. Spontaneous motility of the pig oviduct in vitro. *Biology of Reproduction 26,* 98-104.
Rodriguez-Martinez, H, Garcia, R.B., Ohanian, C., Einarsson, S. 1982b. Histochemical investigation on the distribution of adrenergic nerve terminals in the porcine oviduct. *Journal of Veterinary Medicine A 29,* 64-71.
Rodriguez, H., Kunavongkrit, A. 1983. Chronic venous catheterisation for frequent blood sampling in unrestrained pigs. *Acta Veterinaria Scandinavia. 24,* 318-320.
Roozen, A.W.M., Tsuma, V.T., Magnusson, U. 1995. Effects of short-term restraint stress on plasma concentrations of catecholamines, β-endorphin, and cortisol in gilts. *American Journal of Veterinary Research 9,* 1225-1227.
Statistical Analysis Systems Institute Inc., 1989. SAS/STAT *User's Guide, Version 6*, Fourth Edition, Volume 2, Cary, NC SAS Institute Inc.
Shille, V.M., Karlbom, I., Einarsson, S., Larsson, K., Kindahl, H., Edqvist, L-E. 1979. Concentrations of progesterone and 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ in peripheral plasma during the oestrus cycle and early pregnancy in gilts *Zentralblatt für Veterinärmedizin 26,* 169-181.
Simonsson, A. 1994. Näringsrekommendationer och fodermedelstabeller till svin. *Swedish University of Agricultural Sciences, SLU Info. rapporter*, Husdjur. 75.
Soede, N.M., Helmond, F.A., Schouten, W.G.P., Kemp. B. 1997. Oestrus, ovulation and peri-ovulatory hormone profiles in tethered and loose-housed sows. *Animal Reproduction Science 48,* 133-148.
Soede, N.M., Noorhuizen, J.P.T.M., Kemp, B. 1992. The duration of ovulation in pigs studied by transrectal ultrasonography in not related to early embryonic diversity. *Theriongenology 38,* 653-666
Stott, G.H. 1981. What is animal stress and how is it measured? *Journal of Animal Science 52,* 150-153.
Spilman, C.H., Shaikh, A.A., Harper, M.F.K. 1978. Oviductal motility, amplitude, and ovarian steroid secretion during egg transport in the rabbit. *Biology of Reproduction 18,* 409-417.
Stone, B.A., Seamark, R.F. 1985. Steroid hormones in uterine washings and in plasma of gilts between days 9 and 15 after oestrus and between days 9 and 15 after coitus. *Journal of Reproduction & Fertility 75,* 209-221.
Stroband, H.W.J., Van der Lende, T. 1990. Embryonic and uterine development during early pregnancy in pigs. *Journal of Reproduction & Fertility Supplement 40,* 261-277.
Tarraf, C.G., Knight, J.W. 1995. Effect of uterine space and fetal sex on conceptus development and in vitro release of progesterone and estrone from regions of the porcine placenta throughout gestation. *Domestic Animal Endocrinology 12,* 63-71.

Tienthai, P., Kjellén, L., Pertoft, H., Suzuki, K., Rodriguez-Martinez, H. 2000. Localisation and quantitation of hyaluronan and sulfated glycosaminoglycans in the tissues and intraluminal fluid of the pig oviduct. *Reproduction Fertility Development 12,* 173-182.

Tsuma, V.T., Einarsson, S., Madej, A., Kindahl, H., Lundeheim, N. 1996a. Effect of food derprivtion during early pregnancy on endocrine changes in primiparous sows. *Animal Reproduction Science 41,* 267-278.

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

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

Van der Lende, T., Soede, N.M., Kemp, B.V. 1994. Embryo mortality and prolificacy in the pig. *Principle of pig science 16***,** 297-317.

Van Lier, E., Andersson, H., Pérez Clariget R., Forsberg, M. 1998. Effects of administration of adrenocorticotrophic hormone (ACTH) on extragonadal progesterone levels in sheep. *Reproduction Domestic Animal 33,* 55-59.

Varley, M. and Stedman , R. 1994: Stress and Reproduction. *Principle of Pig Science.* Uni.Press, Nottingham, UK, 277-296.

Vellucci, S.V. 1997a. The autonomic and behavioural response to stress. *In: Stress, Stress Hormones and the Immune System.* John Wiley & Sons, Ltd., 49-70.

Vellucci, S.V. 1997b. Assessment of the Autonomic and Behavioural Effects of Stress. *In: Stress, Stress Hormones and the Immune System.* John Wiley & Sons, Ltd., 157-171.

Viring, S., Einarsson, S., Nicander, L., Larsson, K. 1980. Localisation of the sperm reservoir at the utero-tubal junction of the pig. *Proceeding of the 6th International Congress on Animal Reproduction and Artificial Insemination, 5,* 224-227.

Webel, S.K., Dziuk, P.J. 1974. Effect of stage if gestation and uterine space on prenatal survival in the pig. *Journal of Animal Science 38,* 5, 960-963.

Wilson, M.E., Ford, S.P. 2000. Effect of estradiol-17β administration during the time of conceptus elongation on placental size at term in Meishan pigs. *Journal of Animal Science 78,* 1047-1053.

Acknowledgements

The studies were carried out at the Department of Obstetrics and Gynaecology, Centre for Reproductive Biology, Faculty of Veterinary Medicine, SLU, Uppsala, Sweden.

Financial support was provided by FORMAS (formerly the Swedish Council for Forestry and Agricultural Research [SJFR] and Swedish Meat.

Stig Einarsson, my scientific supervisor, first of all, for giving me the opportunity to work as a PhD-student. You have also always given me complete support, as well as the impression of a never-ending trust in my ability to achieve what was expected (especially at times when my own self-esteem was at an all-time low). Thanks for all the fruitful discussions and for always sparing time for me when I most needed it.

Hans Kindahl, my co-supervisor, for your kind support, encouragement and scientific advice and especially for helping me with all the PG-analyses and interpretation of the results.

Fredrik Hultén, my co-supervisor, for your invaluable help in conquering the SAS-system and for your thorough proofing of my manuscripts, which were always returned to me together with encouraging comments. It was very much appreciated.

Heriberto Rodriguez-Martinez, co-author, for willingly and efficiently sharing your expertise in morphology with me, as well as providing a good critical point of view when revising my manuscripts.

Co-workers, Alfred Mwanza, for teaching me the mystery of ultrasound examinations and catheterising sows, as well as burning the midnight oil together with me during my first confusing year as a PhD-student. Padet Tummaruk, I will always be indebted to you for the tremendous efforts you put into my work, always with a smile and a good sense of humour, making the stressful collecting of material for histological examinations actually enjoyable.

Mats Forsberg for expert advice about hormone analyses.

Lisa Persson for generously sharing her impressive knowledge about morphology with me.

The staff at the labs of the Dept. Of Clinical Chemistry for analysing the blood samples and moreover patiently explaining and showing me how they did it.

Helene Gille, Carola Jansson, Mari Wallbring, Ulrika Mattson, Kjell-Ove Eklund and Ove Gustavsson for taking great care of my sows and for the assistance and enjoyable small-talk before, during and after the operations. Thanks for the much needed and well-appreciated help with heat checking and day and night blood sampling, Monday to Sunday, week after week.

Lotta Sundström, for analysing a considerable amount of my PG-samples and especially for trying to teach me how to analyse PG myself. I will never forget our convivial early mornings in the lab during the cold winter months and all our discussions on more important things than prostaglandins.

Åsa Jansson, for excellent laboratory work and patiently explaining how to do and why, as well as keeping my slides in order.

Birgitta Berner, Helene Gille, Tom Jangby, Bo Fred, Karin Selin and Annika Rikberg for always helping out with a smile and a kind word. Kjell-Åke Ahlin and Kjell-Ove Eklund for helping out with computer-related business.

PhD-students, past and present, at the OG department and especially the old time "Mattåget" and the present Doktorandklubben, for making my working hours enjoyable as well as, with varying frequency, enlightening my life with after-work get-togethers. Bengt and Arne for being good friends and room-mates and having a great sense of humour. Ann-Marie for taking good care of my horses and turning horse-business into a great social event. Ulrika and Ylva, my present room-mates, for putting up with my endless moans and groans during the last hectic year and turning it into a pleasant one, very much thanks to their chatting and gossip.

All other employees and guest researchers, who were great company through the years at OG, it has been varying and interesting, and the pleasure has been all mine.

"Hälsingetjejerna": Maria, Ann-Catrin, Anna-Karin and Agneta, "pumorna" Linda, Fia and Maria, as well as Ann-Sofi, Li, Tina and Annika for all the joys and sorrows we shared over the years, which in different ways brought me to this point. Anna, for your incredible sense of humour that makes life so much more bearable and for dragging me out on skies or to "Friskis". Sandra, Louise and Jenny for caring about and helping me with Shadi and for being trustworthy and fun friends.

My sisters and their families, Ann-Sofie, "the little one" for always being there when I or my family needed you and for never giving up in your task of enlightening me in various subjects. And my "adopted sister", Maria Å. for being my soulmate.

My family, spread out from Jämtland in the north to Gothenburg and Matlock in the southwest for support, encouragement and your concern about "the boys". De två viktigase kvinnorna i mitt liv, mamma Eva och mormor Elin, för att ni alltid har funnits där för mig och gett mig 100-procentigt stöd i allt jag har företagit mig (nästan). And especially to my mother for your total devotion to Max and Leo and for helping us out when we needed it most, your contribution to this book is greater than anybody can imagine.

Alex, my very best and dearest friend and companion, for your complete and utter support in my work and for thorough linguistic proofing of this thesis, it would not have been the same without you, if at all. It is a privilege to share my life with you!

Max and Leo, thinking of you makes me smile, my heart warm and my life bright - you are the best. Big hug!

Populärvetenskaplig sammanfattning

Stress avslöjar sig ofta genom att ett djur inte kan hantera sin omgivning på ett tillfredsställande sätt, vilket även kan leda till att optimala produktionsresultat inte uppnås. Stress är en biologisk reaktion på en händelse som djuret upplever som ett hot mot sin ”jämvikt”. Händelsen definieras som en stressor. Skötselrutinerna i dagens intensiva grisproduktion inkluderar ett antal händelser som kan agera som stressorer för grisarna. Ett sådant exempel är omgruppering av avvanda eller nybetäckta suggor och gyltor. Omgrupperingar leder inte bara till slagsmål för att återupprätta en ny rangordning, utan även till perioder med minskat foderintag för de mer lågrankade djuren.

Det föreligger en stor individuell variation i stresskänslighet bland grisar och det kan därför vara svårt att utvärdera effekten av stress på grisars fruktsamhet och produktivitet. Ett önskvärt mål med stressforskningen är att utröna vad som är skadlig stress för grisarna och när i reproduktionscykeln de är som känsligast, så att man på ett effektivt sätt kan förebygga reproduktionsstörningar orsakade av stress. Syftet med den här avhandlingen var att under kontrollerade former efterlikna den stress som grisen utsätts för vid en omgruppering, samt utvärdera effekterna av denna stress vid två tillfällen under den tidiga dräktigheten.

I de två första studierna utsattes suggor (grisat 2-4 gånger) för två olika stressorer efter ägglossningen under sin andra brunst efter avvänjningen. I den ena studien hade suggorna fri tillgång till vatten, men fick vara utan mat under två dygn (4 utfodringstillfällen), medan de kunde se och höra hur andra suggor fick mat och i lugn och ro åt upp sin ranson. Avsikten var att på ett kontrollerat sätt efterlikna den fasta som de lågt rankade djuren kan utsättas för i en omgrupperingssituation. Försökssuggorna hade signifikant högre blodnivåer av kortisol, prostaglandin $F_{2\alpha}$ -metaboliten och fria fettsyror samt en lägre insulinnivå än kontrollsuggorna. Den del av äggledaren (istmus) som gränsar till livmodern fungerar som en fysiologisk sfinkter och gör att äggen inte tillåts passera ner i livmodern för tidigt. De hormonella förändringar som ägglossningen medför gör att de cirkulära lagren av den glatta muskulaturen runt istmus successivt slappas, vilket leder till en vidgning av äggledaren så att äggen kan passera. Fasta under två dygn ledde till en fördröjd transport av äggen genom äggledaren, troligen som följd av de hormonella förändringarna.

Att injicera någon form av syntetiskt ACTH-preparat är ett av de vanligaste sätten att simulera stress. I den andra studien som utfördes på nyinseminerade suggor gavs upprepade injektioner av Synachten® Depot (tetrakosaktid), ett syntetiskts ACTH, med början 4-8 timmar efter ägglossningen och därefter var 6:e timme under sammanlagt 48 timmar. Suggorna injicerades via en permanent halsvenskateter för att behandlingen i sig skulle vara stressfri. ACTH-behandlingen ledde till en ökad kortisolfrisättning och en initial stegring av progesteron samt en sänkning av prostaglandin $F_{2\alpha}$ -metabolitens basalnivå. De hormonella förändringarna hade ingen signifikant inverkan på äggens transporthastighet genom äggledaren. Hos de ACTH-behandlade suggorna återfanns dock färre spermier bundna till äggens ”hölje” (zona pellucida) och en fördröjd embryonal

delningshastighet jämfört med kontrollsuggorna, vilket tillsammans speglar en negativ förändring av äggledarmiljön.

De följande två studierna koncentrerades till implantationsperioden. Under dag 13 och 14 utsattes inseminerade suggor för samma slags stresspåverkan som tidigare redovisats. Sålunda fick en grupp suggor ingen mat under två dygn medan en annan grupp utsattes för ACTH-stimulering. Suggorna slaktades dag 30 och fostren undersökes både makroskopiskt och mikroskopiskt. ACTH-suggorna hade förhöjda blodvärden av kortisol och insulin under behandlingsperioden. Senare under dräktigheten påvisades en fördröjning i stegringen av östron; från dag 19 till dag 22. Denna fördröjda stegringen av östron hos suggan kan tyda på en omognad hos fostren. Ingen skillnad i den embryonala utvecklingen och fosteröverlevnaden kunde emellertid påvisas hos ACTH-suggorna jämfört med kontrollsuggorna. Hos de fastade suggorna kunde man under behandlingsperioden se förhöjda blodvärden av kortisol och progesteron samt en sänkning av insulin. Vid slakt sågs även förhöjda progesteronkoncentrationer i fostervätskan och dessa nivåer var korrelerade med placentornas storlek; de var i genomsnitt numeriskt större än hos kontrollsuggorna. Detta skulle eventuellt senare under dräktigheten leda till fosterdöd på grund av trängsel i livmodern, sk. ”crowding-effect”. Vid undersökningstillfället (dag 30 i dräktigheten) sågs ingen skillnad i fosteröverlevnad mellan behandlingsgrupp och kontrollgrupp.

Sammanfattningsvis så visar resultaten att en aktivering av suggans ”stress-axel” (hypofys-binjurar) direkt efter ägglossningen leder till en fördröjning av den tidiga embryonalutvecklingen, sannolikt via en förändring av äggledarmiljön. Fasta under denna period leder dessutom till en försening av äggens transport genom äggledaren ner till livmodern. De här förändringarna skulle kunna leda till fosterdöd senare i dräktigheten, på grund av en störd synkroni i samspelet mellan embryonas utvecklingsgrad och "livmoderns hormonella dräktighetsstadium". Fasta och ACTH-stimulering under tiden för ”maternal recognition for pregnancy” hade ingen signifikant effekt på fosteröverlevnaden dag 30 i dräktigheten, men behandlingen hade en signifikant inverkan på suggornas hormonella status och eventuellt även fostrens hormonproduktion under behandlingsperioden. Avsaknaden av signifikanta effekter på fosteröverlevnaden kan dels bero på stora individuella skillnader tillsammans med relativt små försöksgrupper, dels också på att välskötta suggor i god kondition har kapacitet att kompensera för måttlig stresspåverkan, i alla fall efter dag 13 av dräktigheten.