Is there a future for the boar ?

The role of boar stimuli in reproductive processes around estrus in the pig

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Is there a future for the boar ?

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Pieter Langendijk

Proefschrift

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Langendijk, P. Is there a future for the boar ? The role of boar stimuli in reproductive processes around estrus in the pig. This thesis describes several boar stimuli in their potency to elicit estrous behavior and their potency to affect uterine contractility. With different levels of boar stimuli, onset of estrus can be recorded at different time points relative to ovulation, depending on the change in responsiveness of sows to the stimuli. However, the onset of estrus recorded in such ways, as well as the change in responsiveness recorded by using different stimuli to induce estrous behavior, appeared to be bad predictors for the time of ovulation. Time of ovulation is important for the success of insemination. The use of a back pressure test in the presence of a boar appeared to be most appropriate for detection of estrus, as with lower levels of boar stimuli the chance of not detecting estrus increases, and higher levels of stimuli did not add to the efficiency of estrus detection due to habituation. Uterine contractility is increased during estrus and is important for sperm transport, as suppression of uterine contractility reduces fertilisation rate. Stimulation of uterine contractility to a high degree on the other hand, can obstruct the uptake of the inseminate and increase the reflux of semen. Presence of a boar is more potent than 5α-androstenon or tactile stimuli in inducing the release of oxytocin and in stimulating uterine contractility. Moreover, boar presence selectively stimulates uterine contractility only in sows that have a below average uterine contractility, and might therefore be an appropriate way of stimulating uterine contractility during insemination.

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NN08201, 3097

Stellingen

1. There is a future for the boar!
   dit proefschrift

2. In tegenstelling tot wat velen als waarheid beschouwen, 'zuigt' een zeug tijdens de inseminatie het inseminaat niet naar binnen.
   dit proefschrift

3. Voor het positieve effect van de beer op bronstexpressie geldt niet 'hoe meer, hoe beter'. Boven een bepaalde hoeveelheid beercontact treedt adaptatie op van de zeug aan de prikkels van de beer.
   dit proefschrift

4. Het ei was er eerder dan de kip; maar de kip was er eerder dan het kippe-ei.

5. De 'natuur' wordt te gemakkelijk door zowel wetenschappers als niet-wetenschappers als maatstaf voor het denken over welzijn aangehouden.

6. Als de gemiddelde Nederlander beter bekend was met de culinaire mogelijkheden van tapioca, dan was deze zetmeelbron allang verdwenen uit het Nederlandse varkensmenu.

7. De grote aandacht voor voedselveiligheid is eerder het gevolg van een toegenomen bewustzijn dan van een grotere reden tot zorg.

Stellingen behorende bij het proefschrift 'Is there a future for the boar ? – The role of boar stimuli in reproductive processes around estrus in the pig.'
P. Langendijk, 4 dec 2001

Balnea vina Venus corrumpunt corpora nostra,

sed vitam faciunt balnea vina Venus

(Vertaling: Baden, wijn en liefde richten ons lichaam ten gronde,
maar baden, wijn en liefde maken het leven ook)

bron: grafsteen van Claudius Secundus, romeins soldaat
Palazzo Cafarelli, Rome

Voor mekaar

Voorwoord

Zie daar: het proefschrift is klaar!
De punt achter een stuk werk, het begin van nog meer werk (grapje).
Al begint de tand des tijds reeds aan dit proefschrift te knagen
Hoop ik dat de lering nog enige tijd zal dragen.

De afgelopen vier jaar heb ik veel geleerd, en ook veel genoten. En die combinatie is erg belangrijk. Dat die combinatie tot stand kwam, heb ik aan veel mensen te danken. Met Bas en Nicoline heb ik een goed begeleidingsteam gehad, waarbij helicopter-view en gevoel voor detail samensmolten tot een cocktail waar menigeen zijn vingers bij kan aflikken. Fluwelen handschoen- en ijzeren hand-technieken wisten jullie feilloos te combineren tot een werkomgeving waarin ik op een bijzonder prettige manier heb kunnen presteren. Ik heb veel van jullie geleerd, en een goede tijd met jullie gehad. De koek is nog niet op.

Op de vakgroep Veehouderij, later Leerstoelgroep Adaptatiefysiologie, heb ik een gezellige en coöperatieve tijd gehad. Dat is te danken aan de collega's aldaar. Woorden zijn woorden, en daden zijn daden, al dan niet onder een genot van een (vakgroep)borrel. Aan alledrie heb ik goede herinneringen. Allen dank daarvoor.

Ik heb veel tijd op de proefaccomodatie van Zodiac doorgebracht. Daar lag uiteindelijk de fysieke basis van mijn werk. Dat is allemaal goed verlopen, dankzij de flexibele en energieke inzet van een aantal mensen. Hetgeen veel betekent voor het onderzoek, maar ook voor de manier van werken. Ries, Andre, Ben, Truus en Wim, Zeer veel dank!

Het leven is mooi, het is weer dinsdag. Maar niet zonder twee vrienden, die bereid zijn hun kunsten als paranimf op te voeren. Buis en Sven, hartelijk dank.

Salut! Pieter

Contents

General introduction		1
Chapter 1	Effects of boar contact and housing conditions on estrus expression in weaned sows	13
Chapter 2	Responsiveness to boar stimuli and change in vulvar reddening in relation to ovulation in weaned sows	35
Chapter 3	Effects of boar contact on follicular development and on estrus expression after weaning in primiparous sows	55
Chapter 4	Myometrial activity around estrus in sows: spontaneous activity and effects of estrogens, cloprostenol, seminal plasma and clenbuterol	69
Chapter 5	Effects of different sexual stimuli on oxytocin release, uterine activity and receptive behaviour in oestrous sows	91
Chapter 6	Functions of myometrial activity in sperm transport through the genital tract and fertilisation in sows	111
General Discussion		133
Practical implications		162
Summary		165
Samenvatting		171
Curriculum Vitae		177

INTRODUCTION

The role of boar stimuli in reproductive processes around estrus in the pig

Introduction

The male's contribution of gametes to the process of fertilisation is an undisputable role of the boar in the reproductive process in sows. Apart from this, the boar has other functions in the reproductive process which are less well recognised. Estrous behaviour of a sow, like the exhibition of *receptive behaviour* ('standing response') is triggered by sexual stimuli from a boar. Signoret (1970) classified these *boar stimuli* as olfactory, visual, auditory, and tactile. The responsiveness of a sow to such boar stimuli depends on the stage of estrus. The definition of different phases of estrus by the use of different boar stimuli, might be used for correct timing of insemination. Apart from the effect on receptive behaviour, boar stimuli are probably also important in the process of *fertilisation* (Soede, 1993). After insemination, sperm cells have to be transported to the oviducts, a passive process which is believed to depend on the contractile activity of the myometrium. Exteroceptive boar stimuli, like those mentioned above, but also internal stimulation of the genital tract of the sow during mating and components in seminal plasma are believed to stimulate uterine activity. Thus, boar stimuli might have a role in sperm transport and fertilsation. Both the use of boar stimuli to establish behavioral events during the period of receptive estrus and to relate these events to the time of ovulation, as well as the effect of boar stimuli on myometrial activity and subsequent fertilisation are subject of this thesis. In the following sections, these two aspects of reproductive processes around estrus are introduced in more detail, and the central questions of this thesis are outlined.

Boar stimuli and receptive behavior, in relation to the time of ovulation

As outlined above, boar stimuli can evoke receptive behavior in estrous sows. Willemse & Boender (1967) already described that during estrus the responiveness to stimuli initially increases, and then decreases again. Thus, they were able to distinguish two phases during estrus. The 'inseminator period' was defined as the phase during which the sow showed receptive behavior when stimulated manually on the back and in her

flanks (Back Pressure Test: BPT). This phase covered the middle two-thirds of a longer phase during which the sow was responsive to the same BPT, but in the presence of a mature boar; the 'boar period'. Apparently, the BPT alone was less potent in evoking receptive behavior than in combination with boar presence, and the responsiveness of the sows changed in the course of estrus. Depending on the stimuli or combination of stimuli used to evoke estrous behavior, theoretically, numerous phases of estrus can be defined. This concept is illustrated in Figure 1.

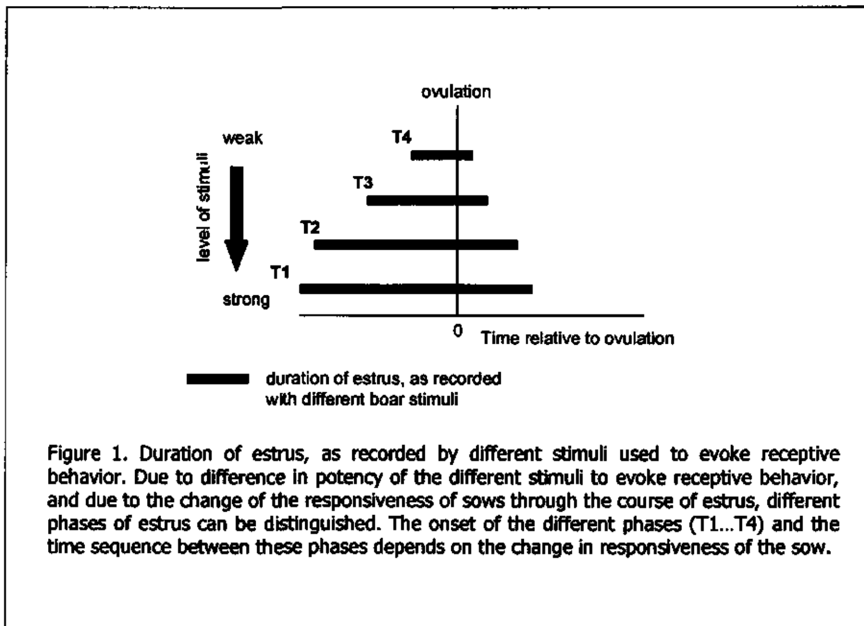

Figure 1. Duration of estrus, as recorded by different stimuli used to evoke receptive behavior. Due to difference in potency of the different stimuli to evoke receptive behavior, and due to the change of the responsiveness of sows through the course of estrus, different phases of estrus can be distinguished. The onset of the different phases (T1...T4) and the time sequence between these phases depends on the change in responsiveness of the sow.

The description of such phases in estrous behavior might be important in order to improve the prediction of the time of ovulation, which is necessary to maximise the chance of fertilisation. Willemse & Boender (1967) suggested on the basis of fertility data that ovulation takes place during the second half of the 'inseminator period'. Studies by Soede & Kemp (1997) and Nissen et al. (1997) confirmed that ovulation takes place two-thirds of the way through the period of behavioral estrus, as recorded using a BPT in the presence of a boar. Knowing this, the duration of estrus can only be used retrospectively to indicate the time of ovulation, and can therefore not be used to predict the correct time for insemination, unless the duration of estrus is a constant

factor. However, the duration of estrus varies considerably between sows (Soede & Kemp, 1997), and therefore the onset of estrus cannot be used to predict ovulation. On the other hand, there might be a fairly constant relationship between the different phases of estrus. If Willemse & Boender (1967) were correct, then the time between the onset of the different phases of estrus should be an indicator for the total duration of estrus, and therefore for the time of ovulation. If, for example, the time between the onset of the 'boar period' and the 'inseminator period' is related to the total duration of the 'boar period, then recording these two events would yield a suitable predictor of ovulation. One aim of this thesis was to study the relation between different phases of estrus detected by using different types of boar stimuli, and the time of ovulation. To satisfactorily predict ovulation for the majority of sows, the predicted time of ovulation should be within a 24-h range, which is the duration of the period of optimal fertilization (Soede et al., 1995). Moreover, a predictor of ovulation is only suitable if it can be obtained in the majority of the sows. The overall responsiveness of sows to boar stimuli (detection rate) used to detect receptive behavior, and factors affecting it, are therefore also important. Hemsworth & Hansen (1990), for example, showed that continuous housing of gilts adjacent to boars reduced the duration of estrus, and therefore the chance of detection. This indicates that the responsiveness of a sow to certain stimuli is not a given fact, but depends on environmental factors, amongst which the stimuli which are used for detection of estrus. In the current thesis, the effects of boar stimuli on the responsiveness of sows to stimuli used to detect receptive behavior are also studied.

Besides behavioral estrus, other characteristics during estrus might be used to predict the time of ovulation. Conductivity of vaginal or cervical mucus, vulvar reddening, and vaginal temperature have been shown to change during estrus (Rojkittikhun et al., 1992; Stokhof et al., 1996). However, to date these 'physical' characteristics have been shown to be too variable to yield a suitable predictor for the time of ovulation, at least according to the criteria mentioned above. In the current thesis, the reddening of the deep inner vaginal mucosa, in contrast to the reddening of the outer vulvar skin normally studied, was also studied in relation to the time of ovulation.

In summary, the first main aims of this thesis are:

To relate the time of ovulation to different behavioural events during the course of estrus, as detected by using different types of boar stimuli. (Chapter 2)

To study the effect of different levels of boar stimuli on the responsiveness of estrous sows to the stimuli used for detection of receptive behaviour. (Chapter 1 and 3)

Boar stimuli around insemination and sperm transport

During mating and artificial insemination in sows, semen is deposited intra-cervically. However, due to the volume, the bulk of semen is flushed directly into the lumen of the uterine body and uterine horns. Therefore, semen depostion is often referred to as intra-uterine. The large volume of the inseminate in both artificial insemination and natural mating are probably needed to flush the semen through the folded structure of the cervix (Figure 2). This is illustrated by the fact that with artificial insemination, the chance of fertilisation decreases when the volume is less than 50 ml (Baker et al., 1968). With semen deposited into the uterine horns, the volume and number of sperm cells needed for successful fertilisation is much smaller (Martinez et al., 2001). From the site of deposition, sperm cells have to be distributed over both horns and have to be transported to the tubal ends of the uterine horns, where they can colonise the oviducts. As the lifespan of sperm cells in the uterine lumen is short compared to the sperm reservoirs in the oviducts, quick transport to the oviducts is essential. This transport is believed to be a passive process, independent of sperm cell motility. Based on the anatomy of the porcine female reproductive tract (Figure 2), it can be hypothesised that transport of the inseminate through the first part of the uterine horns can occur due to the force of gravity. Transport through the latter part of the uterine horns, which proceeds upwards in rostral direction towards the ovaries, mainly seems to depend on myometrial contractions. Individual variation between sows in myometrial activity around insemination might be an important factor explaining individual differences in fertilisation.

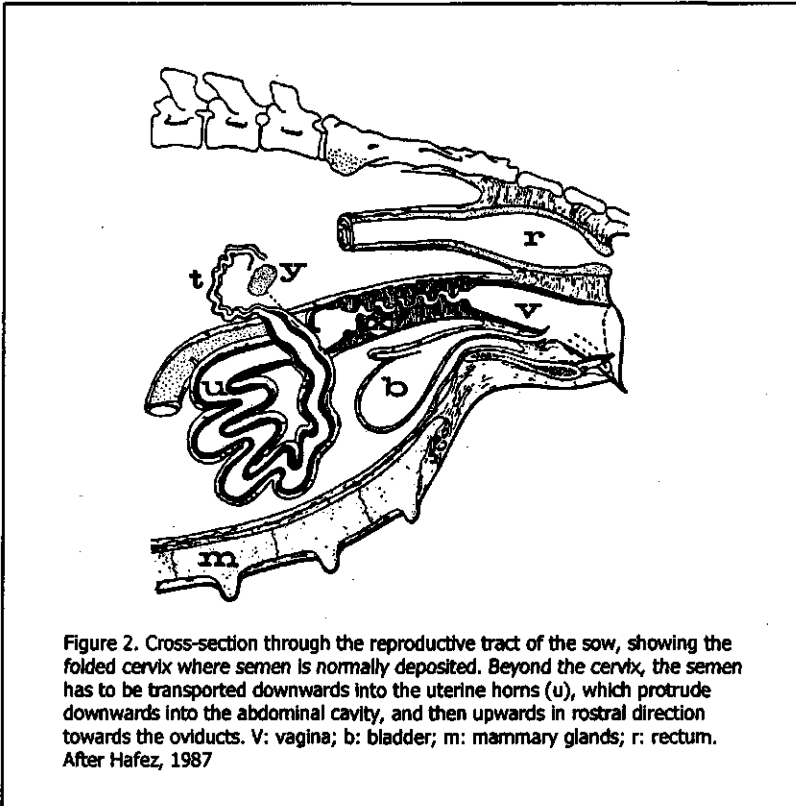

Figure 2. Cross-section through the reproductive tract of the sow, showing the folded cervix where semen is normally deposited. Beyond the cervix, the semen has to be transported downwards into the uterine horns (u), which protrude downwards into the abdominal cavity, and then upwards in rostral direction towards the oviducts. V: vagina; b: bladder; m: mammary glands; r: rectum. After Hafez, 1987

Myometrial activity is an autonomous process, driven by pacemaker activity of the smooth muscles lining the uterus, but can be influenced by external factors, such as boar stimuli and components of seminal plasma. Studies in sows and other mammalian species have shown that myometrial activity is increased during estrus. Variation between sows, however, has hardly been studied. Most studies on myometrial activity have been performed using surgical techniques, with either physical stretch or electrical activity of the myometrium being the parameters studied (Bower, 1974; Scheerboom et al., 1987; Brussow et al., 1988; Claus et al., 1989). A disadvantage of surgical methods is that they can be applied in a limited number of animals, and that they are relatively disturbing to the animals and the way they have to be handled. In the present thesis, a method for non-surgical, uterine intraluminal pressure recording is presented, which was developed by adapting an existing technique used for non-

surgical embryo transfer in pigs (Hazeleger & Kemp, 1984). With this technique it was possible to record uterine pressure changes around estrus in a relatively large numbers of sows, in an 'on farm' situation with relatively small disturbance of the sows, and to study individual variation between sows.

An aim of this thesis was:
To study intraluminal pressure changes around estrus, as an indicator of myometrial activity, and to describe the variation between sows in the change in myometrial activity around estrus. (Chapter 4)

Myometrial activity can be influenced by external factors like seminal plasma and other boar stimuli. Effects of seminal plasma components on myometrial activity have been well described (Claus, 1990). The effect of other components of boar stimuli, such as tactile stimuli and olfactory stimuli, have hardly been studied. Boar presence and mating with a boar has been shown to induce the release of oxytocin into the peripheral blood during estrus (Claus & Schams, 1990). Which components of boar stimuli are responsible for this release is not clear. Boar odor has been shown to induce the release of oxytocin as well (Mattioli, 1986), but other components of boar stimuli have hardly been studied. This thesis presents effects of several components of boar stimuli, other than seminal plasma components, on oxytocin release and on myometrial activity.

An aim of this thesis was:
To study effects of different combinations of boar stimuli on oxytocin release and on myometrial activity, and to study the relationships between boar stimuli, oxytocin release and myometrial activity. (Chapter 5)

Finally, this thesis also contributes to the understanding of the function of myometrial activity in sperm transport and fertilisation. There are several field studies (Schlegel & Loebel, 1971; Konig et al., 1974; Huhn et al., 1977; Pena et al., 1998, 2000) in which addition of smooth muscle stimulating agents, such as oxytocin and prostaglandins, to the inseminate before insemination, causes an increase in the number of live born piglets or the pregnancy rate, or both. In some cases, however, no effects or even

negative effects on fertility parameters are reported (Levis, 2000). The reasons for the varying effects of stimulating myometrial activity around insemination are not clear. Questions like whether there is an optimum in the magnitude of uterine activity, whether the timing of stimulation of uterine activity is important, have not been addressed. In the current thesis, tools for both the suppression and stimulation of uterine activity were tested using the technique for non-surgical pressure recording described above. These tools were used to investigate the effects of suppression and stimulation of uterine activity on sperm cell distribution through the genital tract and on fertilisation.

A final aim of this thesis was therefore:
To study effects of myometrial activity around insemination on sperm transport and fertilisation. (Chapter 6)

The first part of this thesis (Chapter 1 to 3) deals with the relationships between boar stimuli, estrous behaviour and ovulation. The second part of this thesis (Chapter 4 to 6) deals with the function of boar stimuli in the reproductive processes around insemination and fertilisation. In the general discussion, the existing literature and current concepts on these matters are reviewed. The work presented in this thesis is discussed from that perspective, and contributions are made to the existing concepts.

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CHAPTER 1

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Introduction

In sows, standing estrus is expressed by showing a standing response in reaction to boar related stimuli. The period of standing estrus is a basis for the timing of insemination. There are many factors (e.g. parity, season, nutrition) that affect the expression of estrus (Soede and Kemp, 1997). The level of applied boar stimuli affects the chance of evoking a standing response during estrus (Signoret, 1970). Boar stimuli are also important in inducing the onset of ovarian activity and the related estrus behavior. Boar contact can advance estrus in weaned sows by days (Pearce and Pearce, 1992; Walton, 1986). On the other hand, continuous boar contact can reduce the responsiveness to stimuli applied during estrus detection (Tilbrook and Hemsworth,1990; Hemsworth and Hansen, 1990). The stimulus levels applied in a protocol for estrus detection affect the total amount of boar contact per d. Applying extra levels of stimuli in a protocol for estrus detection might therefore affect both the onset of ovarian activity and the responsiveness to boar stimuli during estrus detection.

Group housing vs individual housing after weaning also affects both the onset of ovarian activity and estrus behavior (Pearce and Pearce, 1992). Effects of housing conditions on estrus behavior are variable (Dyck, 1988; Hemsworth et al., 1982; England and Spurr, 1969) and may depend on the amount of social stress received in groups (Pedersen et al., 1993). Whether grouping affects responsiveness to boar stimuli and the onset of ovarian activity in detection protocols with intensive boar contact remains unclear.

The aim of this paper is to study the effect of extra stimulus levels in a protocol for estrus detection and of housing conditions on the responsiveness to detection stimuli and the timing of ovulation. The number of sows expressing a standing response, the onset and duration of standing estrus and the timing of ovulation are described.

CHAPTER 1

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Effects of boar contact and housing conditions on estrus expression in weaned sows

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ABSTRACT

The objective of this research was to study effects of housing conditions and the amount of boar contact in a protocol for estrus detection on estrus detection rate, timing of onset of estrus, duration of estrus and timing of ovulation. After weaning, 130 multiparous sows were assigned to three treatments: HI, in which 52 sows were housed individually in crates and received a high amount of boar contact during estrus detection; HG, in which 52 sows were housed in groups and received a high amount of boar contact; NI, in which 26 sows were housed individually in crates and received a normal amount of boar contact. Estrus detection was performed every 8 h. For each treatment, the standing response to three levels of stimuli was recorded: a back pressure test (BPT) by a man (man-estrus), presence of a teaser boar (spontaneous-estrus) and BPT in the presence of a teaser boar (boar-estrus). In addition, for HI and HG standing response to a fourth level of stimuli was recorded: BPT in a detection-mating area, surrounded by four boar pens (DMA-estrus). To detect ovulation, ultrasonography was performed every 4 h during estrus. Of 117 sows that ovulated, 46 % showed man-estrus, 56 % spontaneous-estrus, 90 % boar-estrus and 97 % DMA-estrus. Mean onset of man-estrus was 107 h (SD 26) after weaning, spontaneous-estrus 106 h (SD 22) after weaning, boar-estrus 99 h (SD 21) after weaning and DMA-estrus 93 h (SD 22) after weaning. Duration of man-estrus was 22 h (SD 14), spontaneous-estrus 29 h (SD 16), boar-estrus 42 h (SD 20) and DMA-estrus 55 h (SD 18). The high amount of boar contact reduced the number of sows showing man-estrus ($P < .05$): 41 % for HG and HI vs 68 % for NI; and reduced duration of boar-estrus ($P < .05$): 43 h for HG and HI vs 52 h for NI. Duration of DMA-estrus for HG and HI was similar to duration of boar-estrus for NI. Onset of estrus and timing of ovulation were not affected by amount of boar contact. Group housing did not affect detection rate and duration of estrus, but did postpone average onset of estrus by ten h, paralleled by a postponement of ovulation. In conclusion, estrus expression is similar at the highest level of stimuli in different protocols for estrus detection. Including higher levels of stimuli in a protocol reduces estrus expression at lower levels of stimuli. This reduction indicates adaptation of sows to a given protocol for estrus detection. Group housing can delay ovulation and related behavioral estrus.

Keywords: Sows, Estrus, Ovulation, Boar, Housing

Introduction

In sows, standing estrus is expressed by showing a standing response in reaction to boar related stimuli. The period of standing estrus is a basis for the timing of insemination. There are many factors (e.g. parity, season, nutrition) that affect the expression of estrus (Soede and Kemp, 1997). The level of applied boar stimuli affects the chance of evoking a standing response during estrus (Signoret, 1970). Boar stimuli are also important in inducing the onset of ovarian activity and the related estrus behavior. Boar contact can advance estrus in weaned sows by days (Pearce and Pearce, 1992; Walton, 1986). On the other hand, continuous boar contact can reduce the responsiveness to stimuli applied during estrus detection (Tilbrook and Hemsworth,1990; Hemsworth and Hansen, 1990). The stimulus levels applied in a protocol for estrus detection affect the total amount of boar contact per d. Applying extra levels of stimuli in a protocol for estrus detection might therefore affect both the onset of ovarian activity and the responsiveness to boar stimuli during estrus detection.

Group housing vs individual housing after weaning also affects both the onset of ovarian activity and estrus behavior (Pearce and Pearce, 1992). Effects of housing conditions on estrus behavior are variable (Dyck, 1988; Hemsworth et al., 1982; England and Spurr, 1969) and may depend on the amount of social stress received in groups (Pedersen et al., 1993). Whether grouping affects responsiveness to boar stimuli and the onset of ovarian activity in detection protocols with intensive boar contact remains unclear.

The aim of this paper is to study the effect of extra stimulus levels in a protocol for estrus detection and of housing conditions on the responsiveness to detection stimuli and the timing of ovulation. The number of sows expressing a standing response, the onset and duration of standing estrus and the timing of ovulation are described.

Materials and methods

Animals

In the period of february to april 1998, one batch of 10 and six batches of 20 multiparous sows (in total 130) arrived at the experimental farm on the day of weaning. The sows were obtained from a commercial farm and consisted of a Yorkshire x Dutch Landrace commercial breed. Litter size averaged 10 and ranged from 7 to 13 piglets; length of lactation was 19 d on average and ranged from 13 to 23 d. The sows received 2.5 kg of a commercial sow diet (12.9 MJ ME kg^{-1}, 130 g CP kg^{-1}) in two portions daily (after estrus detection) and had free access to drinking water during 30 min after feeding. All sows in a batch were housed in the same barn as the four boars that were used for estrus detection. The four boars were commercial AI-boars (Dalland, Merselo, The Netherlands) and 11 mo old at the start of the experiment. The boar pens were located 4 to 8 m away from the sow pens, and separated from the sows by a non-transparent screen to reduce visual, auditory and olfactory contact between sows and boars. Day and night temperature in the barn varied between 16 °C and 22 °C throughout the experiment.

Treatments

Sows were assigned to each of three treatments, with parities (2nd to 12th, 6th parity on average) and body weights (average 233 kg and SD 31 kg) distributed equally among the treatments. The three treatments were: **HI**, in which 52 sows were housed individually and received a high amount of boar contact during estrus detection; **HG**, in which 52 sows were housed in groups and received a high amount of boar contact during estrus detection; **NI**, in which 26 sows were housed individually and received a normal amount of boar contact during estrus detection. The amount of boar contact was determined by the series of stimulus levels applied for each treatment during estrus detection. HG-sows were housed in groups of four and had access to 2.6 m^2 space per sow (one third slatted floors), plus four individual crates where they were

locked up during estrus detection and feeding. HI- and NI-sows were housed in crates with feed mangers (2.2 m x .65 m; one third slatted floors). On average, the distance to the boar pens (6 m) was equal for the three treatments.

Estrus detection

Sows were checked for estrus daily at 0800, 1600 and 2400 from 57 h till 9 d after weaning. For each treatment, the protocol for estrus detection consisted of three stimulus levels. Each stimulus level was applied to all the sows before proceeding with the next stimulus level. Firstly, sows were checked for a standing response in absence of a boar, using the back pressure test (BPT). A BPT consisted of mimicking the tactile stimuli of a boar by pushing the sow in the flanks and rubbing and pressing the sows back to evoke a standing response: a frozen stance, arched back and cocked ears. Secondly, one of the four boars was led in front of the sow pens and the sows were observed for a spontaneous standing response. Thirdly, the BPT was performed in the presence of the boar. In addition, for HG and HI a standing response was recorded to a fourth stimulus level: BPT after having spent 5 min in the detection-mating area (DMA). The DMA was an area of 4.5 x 4.8 m surrounded by the four boar pens designed to maximize boar stimuli during estrus detection (Hemsworth, 1991). In the DMA, sows have visual, auditory, olfactory and head-to-head contact with the boars; the boars remained in their pens during the DMA-procedure and could not mix with the sows. Sows were led into the DMA in groups of four. For HG these were always the same four sows from one group pen; for HI these were always the same four sows from individual crates. The DMA was large enough to avoid aggressive behaviour from other sows and to avoid contact with the boars. HG- and HI-sows were allowed to get used to the DMA-procedure by letting them in the DMA for 15 min on the first two days after weaning. The BPT in the DMA as the fourth stimulus level was only performed for HG and HI. NI-sows were never brought to the DMA. Amount of boar contact was therefore regarded **normal** for NI and **high** for HG and HI.

Depending on the stimulus level used to evoke a standing response, four different, overlapping periods of estrus could be defined. The period during which a standing response was shown to the first stimulus level was defined **man-estrus**. The period during which a standing response was shown to the second level was defined

spontaneous estrus. The period during which a standing response was shown to the third level was defined **boar-estrus** and the period during which a standing response was shown to the fourth level **DMA-estrus**. Onset of an estrus-period was defined as 4 h before first detection of the standing response and end of an estrus period was defined as 4 h after last detection of a standing response.

Ultrasonography

Transrectal ultrasonography (Soede et al., 1992) was performed on d 3 (d 0 is d of weaning) to check for lactational estrus (corpora lutea on the ovary) and on d 8 to check for cystic ovaries, silent estrus (corpora lutea without preceding behavioral estrus) and anestrus (inactive ovaries). From d 3 onwards, ultrasonography was performed every 4 h in sows showing estrus to record timing of ovulation. Timing of ovulation was defined as the average of the last time Graafian follicles were seen and the first time they could not be seen any more on the ovary. Estrus detection, ultrasonography and feeding took approximately 2 h for all sows.

Selection of animals and statistical analyses

Of the 130 sows used in the experiment, four sows (two HG- and two HI-sows) had ovulated during lactation. Seven sows had developed cystic ovaries by d 8 (four HG-sows, three NI-sows). Together with two seriously crippled sows (group HI and group NI), these sows were excluded from the data analyses, leaving 49 HI-, 46 HG- and 22 NI-sows with ovulation for the analyses.

Data were analyzed using SAS (1990). Because not all sows showed standing estrus in response to all stimulus levels and because for NI the fourth stimulus level was not applied, data were unbalanced and could not be analyzed with one model containing both stimulus level and treatment as factors. Therefore, duration, onset and end of estrus were analyzed separately for each stimulus level using the general linear models (GLM) procedure. The basic model $Y_{ij} = \mu + T_i + \beta_1 WEI_j + e_{ij}$, contained the three treatments (T_i) and, except for the onset of estrus, the interval from weaning to estrus (WEI_j) as a covariate. Differences between treatment groups were tested with the LSD-

test, controlling overall type I error. Number of piglets (7-13), batch (1-7) and the interaction T_i x WEI_j did not affect any of the mentioned estrus characteristics and were excluded from further analysis. In contrast, length of lactation appeared to affect duration of boar-estrus and DMA-estrus (b = -1.5 h/d; $P < .05$) and was included as a covariate in analyses of the duration of estrus. Differences between treatments in estrus detection rate were analyzed per stimulus level, using the χ^2-test of the FREQ-procedure. Differences between stimulus levels for onset, duration and end of estrus were analyzed by pooling treatments HI and HG, because NI did not include the fourth stimulus level. LS-means per stimulus level were calculated according to the following model $Y_{ij} = \mu + S_i + L_j + e_{ij}$; S = sow_i, L_j = stimulus level. Differences between treatments in the variance of the onset of estrus were evaluated with the F-test. The difference in detection rate between two stimulus levels was analyzed using the χ^2-test of the FREQ-procedure. Differences in detection rate between stimulus levels were analyzed using the Msign-test of the UNIVARIATE-procedure, comparing two levels at a time.

Results

Estrus detection rate

Applying increasing levels of stimuli during estrus detection increased the number of sows showing a standing response (Table 1). On average, 46 % of the sows showed man-estrus, 56 % of the sows showed spontaneous estrus, 90 % showed boar-estrus and 97 % showed DMA-estrus. The detection rate differed between stimulus levels, except between DMA-estrus and boar-estrus ($P = .06$). The number of sows that showed man-estrus depended on the amount of boar contact sows received per day ($P < .05$); 41% of the sows with high boar contact (HG and HI) showed man-estrus, vs 68% of the sows with normal boar contact (NI). Although not significantly, a high amount of boar contact also decreased the number of sows showing spontaneous estrus. Housing conditions did not have any effect on estrus detection rate at any of the stimulus levels.

Of the 63 sows that did not show man-estrus, 31 sows (49 %) did not show spontaneous-estrus. Of the other 54 sows that did show man-estrus, 20 sows (37 %) did not show spontaneous estrus. The occurrence of spontaneous-estrus was not related to the occurrence of man-estrus ($P > .10$). On the other hand, the occurrence of boar-estrus was related to the occurrence of spontaneous-estrus ($P < .01$). Of the 12 sows that did not show boar-estrus, ten sows did not show spontaneous-estrus, whereas 64 of the 105 sows that did show boar-estrus also showed spontaneous estrus.

Table 1. Number of sows for treatment HI, HG and NI showing estrus at the different stimulus levels.

	Treatment[a]		
	HI	**HG**	**NI**
No. of sows with ovulation	49	46	22
No. of sows showing estrus (%)			
Man-estrus	20 (41)[X]	19 (41)[X]	15 (68)[Y]
Spontaneous-estrus	28 (57)	23 (50)	15 (68)
Boar-estrus	43 (88)	42 (91)	20 (91)
DMA-estrus	46 (94)	46 (100)	-

[a]HI = individually housed sows with high boar contact; HG = group-housed sows with high boar contact; NI = individually housed sows with normal boar contact.

[X,Y] Differences between treatments ($P < .05$) are indicated by different superscripts in one row.

Onset of estrus

Of the sows with high boar contact, four HI-sows and one HG-sow already showed a standing response at the first estrus detection session at least at one of the stimulus levels. As a consequence, onset of estrus could not be estimated for these sows at the concerning stimulus levels.

Table 2. Means and SD per treatment (HI,HG and NI[a]) for the interval from weaning to onset of standing estrus at the different stimulus levels and for the interval from weaning to ovulation.

	Treatment										
	HI			**HG**			**NI**			**Means HI + HG**	
	n	Onset (*h*)	SD	*n*	Onset (h)	SD	*n*	Onset (*h*)	SD	Onset (*h*)	SD
Man-estrus	19	102	17.1	19	113	35.0	15	104	23.2	107^{x}	28
Spontaneous-estrus	28	101	15.2	23	113	29.4	15	104	16.1	106^{x}	23
Boar-estrus	40	94^{x}	14.9	41	105^{y}	26.2	20	$96^{x,y}$	18.2	98^{y}	19
DMA-estrus	43	89	17.8	45	96	25.4	-	-	-	90^{z}	20
Ovulation[c]	48	127^{k}	18.4	45	138^{l}	23.6	22	$132^{k,l}$	20.8		

[a]HI, individually housed sows with high boar contact; HG, group-housed sows with high boar contact; NI, individually housed sows with normal boar contact. [b]Means HI+HG were calculated excluding NI treatment for comparison of stimulus levels. [c]Of 117 sows that ovulated, in two sows timing of ovulation could not be calculated accurately enough. [k,l,m] Different superscripts indicate differences between treatments ($P < .10$, LSD). [x,y,z] Different superscripts in one row indicate differences between treatments (LSD, $P < .05$). Different superscripts in the last column indicate differences between stimulus levels ($P < .05$). Means were calculated excluding animals not showing estrus. In 4 HI-sows and one HG-sow onset of estrus was not detected except for man-estrus.

The onset of estrus was detected earlier with a higher stimulus level (Table 2): on average man-estrus started at 107 h after weaning, spontaneous-estrus at 106 h, boar-estrus at 98 h and DMA-estrus at 90 h after weaning ($P < .01$). The amount of boar contact did not affect onset of estrus (Table 2); for HI onset of estrus was detected at the same time on average as for NI-sows, regardless of the stimulus level applied for estrus detection. In contrast, for group housed sows average onset of estrus was later than for individually housed sows, but this difference was only significant for the onset of boar-estrus (105 h for HG vs 94 and 96 h for HI and NI). The variation in onset of estrus between group-housed sows was higher ($P < .05$) than between individually-housed sows at all stimulus levels (Table 2). The standard deviation of the onset of boar-estrus for HG was 10 h larger than the standard deviation for HI and NI. Within the group-housed sows there were more sows with a retarded onset of boar-estrus (Figure 1). The retarded onset of estrus in group-housed sows was paralleled by a later average timing of ovulation in these sows (138 h after weaning for HG vs 127 h for HI, $P < .05$).

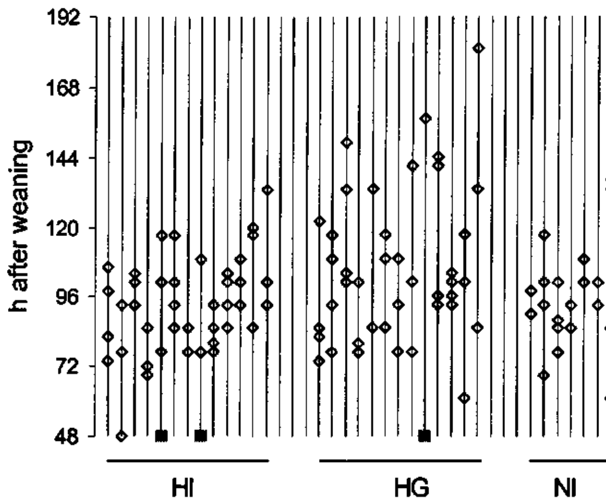

Figure 1. Onset of boar-estrus for individual sows for HI, HG and NI. Vertical lines represent batches. Each symbol represents a sow; for HG and HI sows depicted above each other form the groups as they were brought to the detection-mating area (DMA). Filled squares are sows already showing a standing response at the first detection session. HI = individually housed, high boar-contact; HG = group-housed, high boar-contact; NI = individually housed, normal boar-contact.

Table 3. Means and standard deviation per treatment (HI, HG and NI[a]) for the duration of standing estrus at different stimulus levels.

	Treatment										
	HI			**HG**			**NI**			**Means HI + HG**	
	n	Duration (h)	SD	n	Duration (h)	SD	n	Duration (h)	SD	Duration (h)	SD
Man-estrus	19	24	14.7	19	20	13.7	15	28	10.0	22^{w}	14
Spontaneous-estrus	28	28	14.3	23	30	18.1	15	22	18.6	29^{x}	16
Boar-estrus	40	45^{x}	19.4	41	40^{x}	20.3	20	52^{y}	17.5	42^{y}	20
DMA-estrus	43	55	17.4	45	54	17.9	-	-	-	55^{z}	18

[a]HI, individually housed sows with high boar contact; HG, group-housed sows with high boar contact; NI, individually housed sows with normal boar contact.

[b]Means HI+HG were calculated excluding NI treatment for comparison of stimulus levels.

[w,x,y,z] Different superscripts in one row indicate differences between treatments (LSD, $P < .05$). Different superscripts in the last column indicate differences between stimulus levels ($P < .05$). Means were calculated excluding animals not showing estrus. For one HI-sow and 4 HG-sows end of estrus was not detected except for man-estrus.

Duration of estrus

End of DMA-estrus was not detected for one HI-sow, and the end of one or more estrus periods was not detected for four group-housed sows. For these sows, duration of estrus could not be estimated for use in the analyses at the concerning stimulus levels.

The duration of estrus was minimal 8 h at all stimulus levels and maximal 48 h for man-estrus, 72 h for spontaneous estrus, 88 h for boar-estrus and 96 h for DMA-estrus. The duration of estrus depended on the applied stimulus level ($P = .01$). Average duration of estrus was highest for DMA-estrus (55 h, Table 3) and lowest for man-estrus (22 h). The duration of estrus also depended on the amount of boar contact received by the sows throughout the estrus detection sessions. Sows with a high amount of boar contact (HI and HG) had a lower duration of estrus than sows with normal boar contact (NI), the difference only being significant ($P < 0.05$) for boar-

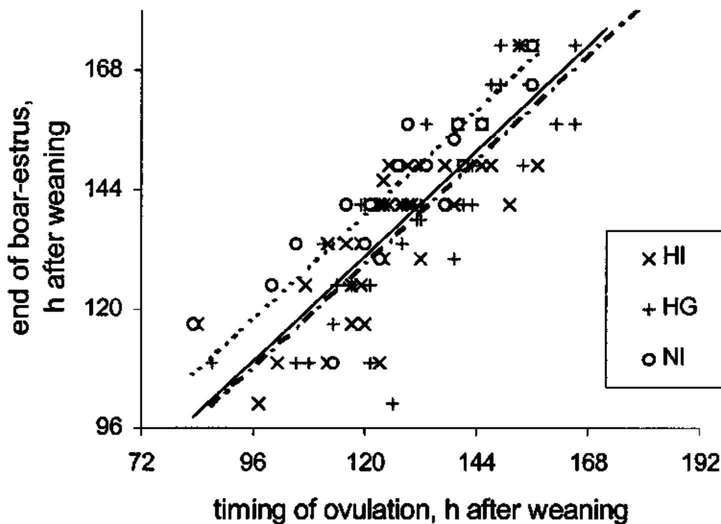

Figure 2. End of boar-estrus in relation to timing of ovulation (ovu) for HI (high boar contact, individually housed; ———), HG (high boar contact, group housed; —·—·) and for NI (normal boar contact, individually housed; -----). End = b_0 + .87 * ovu (R^2 = .73); $b_{0\text{-HI}} = 26^a$; $b_{0\text{-HG}} = 24^a$; $b_{0\text{-NI}} = 34^b$. [a,b] Different superscripts indicate different intercepts (LSD; $P < .05$) for end of estrus.

estrus. Duration of boar-estrus was 45 h for HI and 40 h for HG, compared to 52 h for NI. The duration of DMA-estrus for sows with a high amount of boar contact (55 h on average) was similar to the duration of boar-estrus for sows with normal boar contact (52 h). The duration of estrus was not affected by housing conditions.

Comparing animals with the same timing of ovulation, there was no difference in the onset of estrus between the three treatment groups. In contrast, the high amount of boar contact advanced the ending of boar-estrus by 8 h for HI-sows and 9 h for HG-sows ($P < .05$), compared to NI-sows (Figure 2). The timing of ovulation as a percentage of the duration of boar-estrus was 82 % in sows with normal boar contact, but 90 % for HI and 95 % for HG. Excluding sows with a duration of boar-estrus of 8 h, the relative timing of ovulation was 66 % for NI, 77 % for HI and 81 % for HG. At the other stimulus levels, there was also an advancement of the ending of estrus for sows with a high amount of boar contact, but this was not significant.

Discussion

This study confirms earlier results that a higher level of boar stimuli during estrus detection increases the chance of evoking a standing response. The number of sows that responded positively to the BPT in the DMA or in presence of the boar was higher than the number of sows responding to lower stimulus levels. Signoret (1970) showed that combining individual elements of boar stimuli (olfactory, auditory, visual and tactile), increases the number of animals showing a standing response. It was not clear whether the presence of the boar (without BPT) was a stronger stimulus than the back pressure test by the man. Man-estrus did not differ from spontaneous-estrus in detection rate, onset or duration. Nonetheless, these stimulus levels were not comparable, as the occurrence of spontaneous-estrus in individual sows was not related to the occurrence of man-estrus. In the present study, standing estrus was recorded earlier and lasted longer at higher stimulus levels, indicating that around the onset of estrus, the responsiveness to boar stimuli increases. This was also found by Willemse and Boender (1967), who recorded a period of man-estrus which was preceded by a period during which sows only showed a standing response in presence

of a boar. The variation in responsiveness between individual sows was much larger than the difference between stimulus levels. Of the sows showing estrus, the minimal duration of standing estrus was 8 h at all stimulus levels; the maximum duration of estrus ranged from 48 h for man-estrus to 96 h for DMA-estrus. The difference in duration of estrus between the lowest and the highest stimulus level was only 30 h on average. A large range in the duration of boar-estrus was also recorded by Soede et al. (1996; 24 to 88 h) and Weitze (1996; 32 to 96 h). Steverink et al. (1999) found an average duration of estrus per farm ranging from 31 to 64 h, in a study on 55 farms. The variation between farms accounted for 23 % of the total variation in the duration of estrus. Use of different stimulus levels during estrus detection probably contributes to the variation in the duration of estrus between farms and studies. Other factors like breed of the sows, parity and stress, also contribute to variation in responsiveness of sows to estrus detection stimuli (Soede and Kemp, 1997).

In the present experiment, a high amount of boar contact reduced the percentage of sows showing man-estrus from 68% (HI and HG) to 41% (NI). Data in literature on estrus detection rate in absence of a boar range from 40% (Pearce and Pearce, 1992) to 92% (Soede, 1990). Differences between studies in detection rate might be caused, amongst other factors, by the amount of boar contact sows received. The lower detection rate for sows with a high amount of boar contact suggests that boar contact can reduce the responsiveness to lower stimulus levels. Pearce and Pearce (1992) and Hemsworth and Hoogerbrugge (1982) showed that boar contact for ten min daily increased the number of sows expressing standing estrus in absence of a boar as compared to no boar contact. For HG and HI intensive boar contact occurred three times daily during 10-15 min. Overstimulation of HG- and HI-sows might be an explanation for the reduced responsiveness to lower stimulus levels. The high amount of boar contact also resulted in a lower duration of standing estrus for HI and HG; duration of boar-estrus was 45 h for HI and 40 h for HG vs 52 h for NI. Strikingly, the duration of DMA-estrus for HI (55 h) and HG (54 h) was similar to the duration of boar-estrus for NI (52 h). Estrus expression in reaction to the BPT in the DMA (HG and HI) was not higher compared to the BPT in presence of a boar for NI. End of boar-estrus was advanced ($P < .05$) for HG and HI, although onset of boar-estrus for these treatments did not differ from NI. Responsiveness of sows to stimuli in a protocol for

estrus detection seems to adapt to the amount of boar contact. The total amount of boar contact sows received during estrus detection had a remnant effect on the responsiveness to stimuli in later detection sessions. As a consequence, responsiveness to lower stimulus levels was reduced towards the end of estrus for HG and HI, compared to NI. The carry-over effect of earlier boar contact might be due to habituation to boar stimuli (desensitation) or due to the development of expectation in sows with high boar contact. In the latter case estrus expression in reaction to lower stimulus levels would be reduced in sows anticipating an extra stimulus level (DMA). There is not much literature on the effect of boar contact on the duration of estrus in weaned sows. Only Hemsworth and Hansen (1990) observed a shorter duration of estrus (.2 d shorter) in sows housed adjacent to a boar instead of housing away from the boar, suggesting habituation to boar stimuli. Caton et al. (1986) found a negative effect of continuous boar contact on the induction of puberty in gilts, which was counteracted by exposing the gilts to a different boar 15 min daily.

Several studies have found an advancing effect of boar contact on the onset of estrus in weaned sows of .4 to 4 d, with average weaning to estrus intervals in these studies of more than a week (Pearce and Pearce, 1992; Walton, 1986; Hemsworth et al., 1982). In our study, a high amount of boar contact did not advance the onset of estrus when compared at one level of detection stimuli. The contrast with earlier studies may be explained by the fact that in the present study high boar contact was opposed to boar contact three times daily, instead of comparing short daily boar contact to the absence of boar contact in earlier studies. As pointed out earlier, continuous boar contact can even suppress the expression of estrus behavior in weaned sows, without altering the timing of onset of estrus (Hemsworth and Hansen, 1990). Moreover, the average interval from weaning to estrus in our study was only 4 d, which might explain the lack of advancement of the onset of estrus by increasing the amount of boar contact. The present data indicate an effect of the amount of boar contact on the responsiveness of sows to stimuli during estrus detection without altering ovarian events and the onset of estrus. Regulation of estrus behavior, however, is complex and the effect of boar contact depends on many other factors like boar preference, boar libido, housing conditions and conditions during estrus detection (Soede and Kemp, 1997). Stress, as a consequence of the extra handling of sows in treatments HI and

HG might have been an explanation for the reduced responsiveness during estrus detection in these treatments. Daily acute stressing during the days before and during estrus, however, did not affect the duration of estrus in gilts (Turner et al., 1998). If the extra handling of HI- and HG-sows, by bringing them to the DMA and back, was perceived as a stressor, it was probably too short to affect estrus behavior. The presence of the four boars in the DMA was not likely to pose any threat to the sows because there was enough space to either search or avoid contact with the boars.

Housing conditions did not affect detection rate and duration of estrus at any stimulus level. Barnett and Hemsworth (1991) found a slight difference in estrus detection rate (*100 % in groups vs 96 %, $P < .07$*) between *group housed sows* (6 sows per group, 2.1 m^2 per sow) and individually penned sows. England and Spurr (1969) found no difference between group housing (8 to 12 sows per group, 3 m^2 per sow) and individual penning in detection rate. Group housing can either be stimulatory or detrimental to sexual behavior in gilts, depending on group size (Barnett et al., 1986) and space allowance (Hemsworth et al., 1986). Small and large groups cause more stress than moderately sized groups (4 to 8 sows). In the present study sows had access to at least 4 m^2 (including feeding crates) per sow, which should be enough to minimize negative effects of space allowance (Hemsworth et al., 1986). According to Arey and Edwards (1998) and Pedersen (1993) aggression between sows is concentrated within two days after grouping and a social rank is established within this period. Pedersen et al. (1993) found that in groups, low ranking sows show less sexual behavior in that they mount less frequently, show less levering of other sows and have less nose/body contacts. Moreover, lower ranking sows have a later onset of estrus and have a shorter duration of estrus than dominant sows. However, these authors did not compare group housing with individual housing. Recording of aggression between sows in the DMA and in the group pens on the first two days after arrival (data not shown) yielded a very low number of interactions between sows. As a consequence, social rank could not be calculated for all groups in this study. In groups where a rank was established, no relation between social rank and onset or duration of estrus was found.

Group housing did not advance the onset of estrus in this study. In contrast, average onset of estrus was postponed in group housed sows, being significant only for boar-estrus. The later onset of estrus for HG was apparently caused by a large range between the first sow showing estrus and the last sow showing estrus. Literature on the effects of group housing on the onset of estrus is equivocal. Group housing has been found to either advance the onset of estrus by days (Hemsworth and Hoogerbrugge, 1982; Pearce and Pearce, 1992) or to have no effect on the onset of estrus. The average onset of estrus in these studies was more than 10 d after weaning. The average interval from weaning to onset of estrus in our study (4 d) might have been too short to be advanced by group housing. In our study the postponement of the onset of estrus in group housed sows was paralleled by a delay in the average timing of ovulation in these sows. This indicates that group housing might have delayed the cascade of endocrinological mechanisms responsible for pre-ovulatory ovarian events and the onset of estrus behavior. Nevertheless, group housing did not affect duration of estrus. As pointed out before, social rank might not have been a social stressor in the present experiment, but continuous mounting and derangement by other sows coming into estrus might have been responsible for the postponement of the onset of estrus in group mates. Hormones related to social stress (ACTH and corticosteroids) have been shown to delay ovarian events and ovulation in sows (Arey and Edwards, 1998).

Implications

This study shows that the total amount of boar contact is one of the factors that affect the responsiveness to boar stimuli. In a given protocol for estrus detection, response to stimuli is maximal at the highest stimulus level. However, including an extra (higher) stimulus level does not necessarily increase estrus expression. Sows adapt their responsiveness to the highest stimulus level, resulting in a suppression of the responsiveness to lower stimulus levels. The amount of boar contact might therefore affect the recorded duration of estrus and have consequences for insemination based on standing estrus. Group housing postponed the onset of estrus after weaning, probably by delaying the sequence of ovarian events. This indicates that social stress

might affect onset of ovarian activity and the related estrus behavior. More research has to be conducted to study factors related to estrus expression in sows.

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CHAPTER 2

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Responsiveness to boar stimuli and change in vulvar reddening in relation to ovulation in weaned sows

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ABSTRACT

In 117 weaned sows, changes in estrous behavior and vulvar reddening were related to timing of ovulation. Detection of estrus was performed every 8 h with four levels of boar stimuli to record the change in responsiveness to these stimuli. This resulted in four overlapping phases of estrus, during which a standing response could be evoked: man estrus (standing response to back pressure test, BPT, in absence of a boar), spontaneous estrus (standing response in presence of a boar, no BPT), boar estrus (standing response to boar + BPT), and DMA estrus (BPT in presence of four boars). In addition to the detection of estrus, the change in reddening of the inner vulvar mucosa was recorded. Manifestation of estrus in response to the four stimuli (BPT, boar, boar + BPT, DMA + BPT) occurred in 46, 56, 90, and 97 % of the sows, respectively. Onset of the four phases occurred 24 h (SD 13 h), 23 h (SD 15 h), 34 h (SD 13 h), and 41 h (SD 12 h) before ovulation. The duration of the intervals between the different phases of estrus explained 10 to 50 % of the variation in the timing of ovulation relative to the onset of the phases. However, these intervals could not be calculated for all sows because estrus was not expressed at every stimulus level by each sow. End of vulvar reddening occurred on average 21 h (SD 14 h) before ovulation. Except for five sows that ceased to show vulvar reddening within 5 h after ovulation, end of vulvar reddening occurred before ovulation, within a 70-h range. Of the sows showing boar estrus, 90% also showed vulvar reddening. For sows that showed vulvar reddening until after onset of boar estrus (two thirds of the sows), the end of reddening occurred within a much smaller range: from 36 h before until 2 h after ovulation. Onset of estrus, regardless at which stimulus level it is detected, appears too variable relative to timing of ovulation to be used as a predictor for ovulation. Duration of the different stages of responsiveness explains only some of this variation and cannot be obtained on all sows. Combining information on vulvar reddening and boar estrus can predict ovulation within a reasonable range for two thirds of the sows.

Keywords: Sows, Estrus, Ovulation, Boar

Introduction

In sows, optimal results in terms of fertilized oocytes are achieved when artificial insemination takes place in the period of 24 h before ovulation (Soede et al., 1995). Prediction of ovulation is therefore a necessity in AI strategies focused on maximal fertilization with a low number of inseminations. Onset of estrus is a poor predictor because of the large variation in its timing relative to ovulation (Soede and Kemp, 1997). Duration of estrus is a fairly good indicator of ovulation, because it takes place approximately two thirds of the way through the period of behavioral estrus (Nissen et al., 1997). However, duration of estrus cannot be assessed in advance of ovulation.

In the course of estrus, responsiveness to stimuli applied to evoke estrous behavior (standing response) increases and then decreases again. Willemse and Boender (1967) divided the period of estrus into three stages, based on the responsiveness to either a man (man estrus) or a boar (boar estrus) as stimuli for detection of estrus. Man estrus covered the middle two thirds of boar estrus. The authors did not detect ovulation, but insemination results suggested that it took place during the second half of man estrus. Applying additional levels of stimuli during detection of estrus might distinguish more phases of responsiveness, and yield a more accurate predictor of ovulation. In addition to behavioral characteristics, physical characteristics might yield a predictor of ovulation. Vaginal temperature and conductivity of vaginal mucus are poorly related to the timing of ovulation (Stokhof et al., 1996; Soede et al., 1997). In the current study, changes in behavioral estrus and vulvar reddening were monitored, in order to establish a suitable predictor for ovulation.

Materials and Methods

Animals and housing

Data on estrus and ovulation in the current study were obtained from an experiment described by Langendijk et al. (2000). The experiment was approved by the Wageningen University ethical commission on animal experiments, and was conducted

between February and May 1998. In one group of 10, and six groups of 20, multiparous sows (total 130) arrived at the experimental farm on the day of weaning, at 2 wk intervals. The sows were obtained from a commercial farm, and consisted of a Yorkshire x Dutch Landrace commercial breed. Litter size averaged 10 and ranged from 7 to 13 piglets; length of lactation was 19 d on average and ranged from 13 to 23 d. At the experimental farm, sows received 2.5 kg of a commercial sow diet (12.9 MJ ME kg^{-1}, 130 g CP kg^{-1}) in two portions daily (after detection of estrus) and free access to drinking water during 30 min after feeding. Seventy-eight sows were housed individually in crates (2.2 m x .65 m), and 52 sows were housed in groups of four. The group housed sows had access to 2.6 m^2 per sow, plus four individual crates in which they were locked up during detection of estrus and feeding. The four boars that were used for detection of estrus were housed in the same barn as the sows. These were commercial AI boars (Dalland, Merselo, The Netherlands), 11 mo of age at the start of the experiment. The boar pens were situated at a distance of at least 4 m from the sows, and separated from the sows by a non-transparent screen which reduced visual, auditory and olfactory contact.

Detection of estrus and boar stimuli

Sows were checked for estrus daily at 0800, 1600, and 2400 from 57 h until 8 d after weaning. Before detection of estrus started, reddening of the deep inner vaginal mucosa was judged in all sows by spreading the vulvar lips. Both detection of estrus and judging of vulvar reddening were performed by one of two experienced persons, according to a precisely defined protocol. Reddening was scored as 0 (pale), 1 (pink), 2 (red) or 3 (dark red). Onset of vulvar reddening was defined as 4 h before the first time a score of minimal 2 was given, and the end of vulvar reddening was defined as 4 h after the last time a score of minimal 2 was given. Every time detection of estrus was conducted, sows were checked for a standing response in reaction to a set of increasing levels of boar stimuli. Firstly, all sows were checked for a standing response in absence of a boar, using the back pressure test (BPT). A back pressure test lasted ca 20 s, and consisted of mimicking the tactile stimuli of the boar by pushing the sow in the flanks and rubbing and pressing the sows' back. If the sow reacted with a frozen stance, arched back and cocked ears, this was recorded as a standing response.

Secondly, approximately 15 min after the BPT, one of the four teaser boars (randomly chosen) was led in front of the crates and group pens. The teaser boar was restricted to four sows at a time and the sows were observed for a spontaneous standing response (within 30 s). Thirdly, the BPT was performed on each sow, in the presence of the teaser boar, immediately after having observed for the spontaneous standing response. Finally, a fourth stimulus level was applied to 104 of the 130 sows, approximately 25 min after the third stimulus level. These sows were led into a detection mating area (DMA) in fixed groups of four. The DMA is an area of 4.5 x 4.8 m surrounded by four boar pens, designed to maximize boar stimuli during detection of estrus (Hemsworth, 1991). In the DMA, sows have visual, auditory, olfactory and head-to-head contact with the boars, and can interact with each other. After 5 min in the DMA, the sows were checked for a standing response by a BPT. To check whether the extra boar stimulation in the DMA affected estrus expression, 26 sows received only the first three levels of stimuli during detection of estrus. On the day of arrival (d 0) and on d 1, the 104 sows that were also submitted to the fourth stimulus (DMA), were allowed into the DMA during 15 min, to adapt to the procedure.

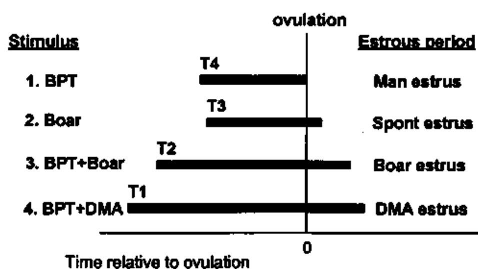


Figure 1. The four subsequent stimuli used for detection of estrus in the experiment (BPT, Boar, BPT+Boar, and BPT+DMA), and the four phases of estrus during which sows responded to these stimuli with a standing response (man estrus, spontaneous estrus, boar estrus, and DMA estrus). BPT: back pressure test. Boar: presence of a teaser boar. DMA: detection-mating area, presence of four boars. Intervals between estrous phases were defined $Int_{DMA\text{-}Boar}$ (T1 to T2), $Int_{DMA\text{-}man}$ (T1 to T4), $Int_{DMA\text{-}spont}$ (T1 to T3), $Int_{boar\text{-}man}$ (T2 to T4), $Int_{boar\text{-}spont}$ (T2 to T3), and $Int_{spont\text{-}man}$ (T3 to T4).

Depending on the stimulus level used to evoke a standing response, four different, overlapping phases of estrus could be defined (Figure 1). These phases were defined **man estrus, spontaneous estrus, boar estrus**, and **DMA estrus**, for the four levels of stimuli, respectively. Onset of a phase was defined as 4 h before the first detection of the standing response, and end of a phase was defined as 4 h after last detection of a standing response. Onset of the different phases were defined T1, T2, T3, and T4 (Figure 1). The interval between the onset of DMA estrus and the onset of boar estrus (T1 to T2), was defined $\mathbf{Int_{DMA\text{-}boar}}$. The intervals between the other phases were calculated similarly: $\mathbf{Int_{DMA\text{-}man}}$ (T1 to T4), $\mathbf{Int_{DMA\text{-}spont}}$ (T1 to T3), $\mathbf{Int_{boar\text{-}man}}$ (T2 to T4), $\mathbf{Int_{boar\text{-}spont}}$ (T2 to T3), and $\mathbf{Int_{spont\text{-}man}}$ (T3 to T4).

Ultrasonography

Ovarian condition was checked by transrectal ultrasonography, using a 7.5 Mhz annular array sector probe (Pie Medical, Maastricht, The Netherlands). The method of ultrasonography was developed at our lab (Soede et al., 1992), and operated by trained and experienced staff. Ultrasonography was performed on d 3 (d 0 is day of weaning) to detect lactational estrus (corpora lutea on the ovary), and on d 8 to detect cystic ovaries (follicles > 10 mm), inactive ovaries (no follicles > 4 mm), or silent estrus (ovulation without signs of estrus). From d 3 onwards, ultrasonography was performed every 4 h in sows showing estrus in order to record timing of ovulation. Time of ovulation was defined as the time between the last time preovulatory follicles were detected and the first time no follicles could be detected on the ovary. The first time follicles could not be detected on the ovary, diagnosis was always double-checked by a second person. Detection of estrus, ultrasonography, and feeding took approximately 2 h.

Characteristics of estrus and statistics

The timing of ovulation was expressed in hours from an estrous characteristic such as onset of a phase of estrus, or end of vulvar reddening. Timing of ovulation relative to onset of man estrus, spontaneous estrus, boar estrus, DMA estrus, and onset of vulvar reddening was normally distributed (not shown). The variation in timing of ovulation

relative to the different characteristics was therefore expressed by the SD of the uncorrected means. To test the hypothesis that length of the intervals between phases of estrus was related to the time from onset of a phase to ovulation, SD (Mean Square Error) was also calculated after correcting for length of the intervals:

$Y_{ij} = \mu + \beta_1.Int_i + e_{ij}$ (Y_{ij} is ovulation in hours after onset of man-, spontaneous-, boar-, or DMA estrus; Int_i is length of an interval between two phases of estrus).

The GLM procedure of SAS (SAS Inst. Inc., Cary, NC) was used to analyse the model. For some sows, housing, and detection of estrus (DMA), was in groups of four sows. Nevertheless, data on estrus were analyzed for each sow individually, because the aim of the study was to estimate timing of ovulation, given the variation caused by different factors like group effects. Exclusion of group effects was justified by preliminary analyses, which showed that there was no effect of groups on any of the analyzed characteristics. The housing conditions and the extra boar contact received by the sows that went through the DMA-procedure, did not affect any of the data studied in this paper (Langendijk et al., 2000). Therefore, these were not included in the model. Interval from weaning to estrus was related to duration of estrus ($P <$ 0.05), but did not affect the relation between the timing of ovulation and the length of Int_i, for any of the phases. Therefore, the interval from weaning to estrus was excluded from the model. Because most animals showed both boar estrus and vulvar reddening, these two characteristics were combined to predict timing of ovulation. Three categories of sows were defined: sows that showed no vulvar reddening until after onset of boar estrus, sows that showed vulvar reddening at onset of boar estrus, and sows that had ceased to show vulvar reddening before onset of boar estrus. Timing of ovulation in these categories was expressed in hours after onset of boar estrus and in hours after end of vulvar reddening. Differences in standard deviation between different estimations of ovulation were tested with the F-statistic. Normality of parameters was tested in the UNIVARIATE procedure of SAS, with the Shapiro-Wilk statistic. Correlations between variables were calculated according to Pearson using the CORR procedure of SAS.

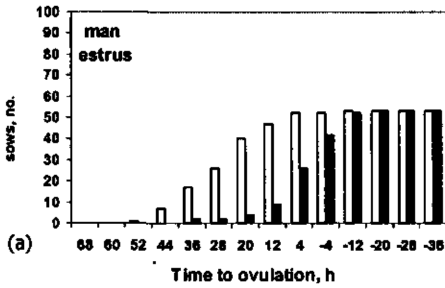
Results

Onset of estrus in relation to ovulation

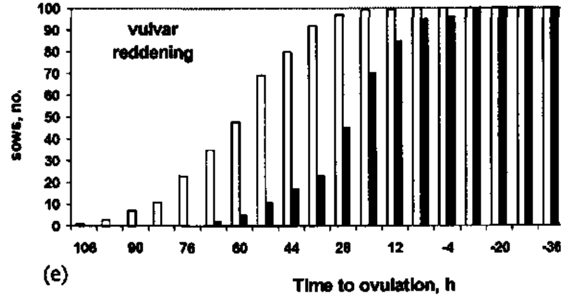
Of the 130 sows used in the experiment, four sows had ovulated during lactation, seven sows had developed cystic ovaries by d 8, and two sows were too crippled to complete the experimental period. These sows were excluded from further analyses, leaving 117 sows that ovulated. Of these 117 sows, 95 sows had been submitted to four stimulus levels, and 22 sows had been submitted to three stimulus levels. Of the 117 sows, one sow did not show standing estrus at any of the stimulus levels, and two sows already showed estrus at the first time detection of estrus was conducted. For these three sows time of ovulation could only be related to vulvar reddening. The number of sows detected in estrus differed between stimulus levels. Of the 117 sows with ovulation, 46, 56, and 90 % showed man-, spontaneous-, or boar estrus; of the 95 sows that were submitted to the DMA-stimulus-level, 97 % showed DMA estrus. On average, duration of man-, spontaneous-, boar- and DMA estrus was 24 h (SD 13 h), 27 h (SD 17 h), 44h (SD 20 h), and 55 h (SD 18 h) respectively.

Onset of behavioral estrus occurred before ovulation at all of the stimulus levels (Figure 2), except for one sow that ovulated before onset of man estrus, and for two sows that ovulated before onset of spontaneous estrus. DMA estrus was recorded 41 h (SD = 12 h) before ovulation on average, followed by boar estrus (34 h; SD = 13 h), man estrus (24 h; SD = 13 h) and spontaneous estrus (23 h; SD = 15 h). On average, intervals from the onset of man estrus to ovulation and from spontaneous estrus to ovulation were similar. Within each stimulus level, onset of estrus relative to ovulation was very variable between sows. The range between the first and the last sow ovulating after onset of one of the phases of estrus, was between 48 h (man estrus) and 64 h (boar estrus) (Figure 2).

Reddening of the inner vulva, as a sign of proestrus, started 52 h (SD = 18 h) before ovulation and was shown by 87 % of the sows. Except for five sows which still had a reddened vulvar mucosa until 5 h after ovulation, a score red was never observed after ovulation (Figure 3). End of vulvar reddening occurred 21 h (SD = 14 h) before



(a)



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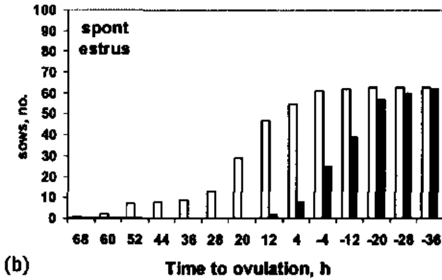
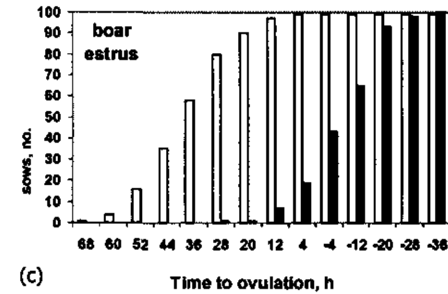


(b)



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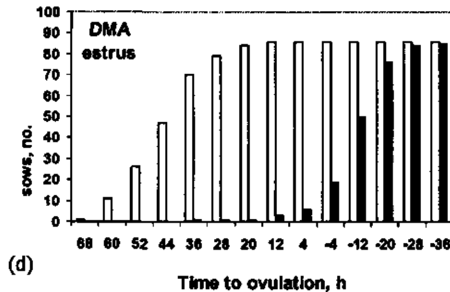

(d)

☐ onset detected ■ end detected

Figure 2. Cumulative number of sows in which onset of standing estrus (☐), and end of standing estrus (■) was recorded for man estrus (a), spontaneous estrus (b), boar estrus (c) and DMA estrus (d), relative to the timing of ovulation. Man estrus, spontaneous estrus, boar estrus and DMA estrus were those phases of estrus, during which sows showed a standing response to a back pressure test, presence of a boar, back pressure test in presence of a boar, or back pressure test in presence of four boars, respectively. Figure (e): cumulative number of sows in which onset of vulvar reddening (☐) and end of vulvar reddening (■) was recorded, relative to the timing of ovulation. Vulvar reddening was scored as the reddening of the internal vulvar mucosa.

ovulation, but this timing was also very variable. The interval from the end of vulvar reddening to ovulation was not normally distributed ($P < 0.01$), but skewed. The variation in timing of ovulation relative to the end of vulvar reddening was smaller ($P < 0.05$) for sows which showed a reddened vulva for a longer period of time (Figure 3); SD was 9.5 h in sows which had a reddened vulva for more than 40 h, compared with a SD of 14 h in sows which had a reddened vulva for 40 h or less. The duration of vulvar reddening was 36 h in sows that ovulated, ranging from 0 to 96 h.

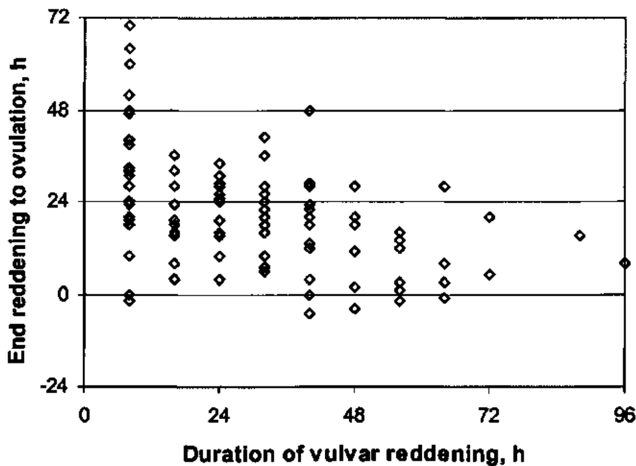

Figure 3. Timing of ovulation in hours from the end of vulvar reddening, in relation to the duration of vulvar reddening.Vulvar reddening was scored by opening the vulvar lips, and observing the inner vulvar mucosa.

Intervals between phases of estrus in relation to timing of ovulation

There was large variation in the length of the intervals between different phases of estrus (SD 11 h to 20 h, Table 1). Onset of DMA estrus preceded or coincided with the onset of boar estrus for 68 of 76 sows, preceded or coincided with onset of spontaneous estrus for 46 of 47 sows, and preceded or coincided with onset of man-

oestrus for 33 of 36 sows. Onset of boar estrus preceded the onset of spontaneous estrus in all sows and man estrus in 45 out of 49 sows. However, in 15 out of 33 sows onset of spontaneous estrus occurred after onset of man estrus.

Table 1. Mean duration (h) of the intervals between onset of different phases of estrus. A phase of estrus was defined as the period during which a standing response was exhibited to either a back pressure test (man estrus), presence of a boar (spont estrus), back pressure test (BPT) in presence of a boar (boar estrus), or BPT in presence of four boars (DMA estrus).

Interval[1]	n	Mean, h	SD, h	Minimum	Maximum
$Int_{spont\text{-}man}$	33	1	20	-40	48
$Int_{boar\text{-}man}$	49	11	17	-40	56
$Int_{DMA\text{-}man}$	36	21	17	-8	56
$Int_{boar\text{-}spont}$	59	15	11	0	40
$Int_{DMA\text{-}spont}$	49	19	13	-8	48
$Int_{DMA\text{-}boar}$	78	8	11	-16	32

[1] $Int_{spont\text{-}man}$ is the interval between onset of spont-estrus and onset of man-estrus; $Int_{boar\text{-}man}$ is interval between onset of boar-estrus and onset of man-estrus; etc.

For all phases of estrus, the timing of ovulation after onset of estrus was related to length of the intervals between the different phases (Table 2). Length of the different intervals explained 10 to 50 % of the variation in the estimated timing of ovulation. A 24 h change in the length of an interval was related to a 7 to 19 h change ($P < 0.01$) in the estimated timing of ovulation. Depending on the interval regarded, timing of ovulation relative to onset of estrus was related either positively or negatively to length of the interval. The SD of the timing of ovulation was not significantly lower than when timing of ovulation was related to the onset of estrus only (Table 2).

Combination of boar estrus and vulvar reddening to predict ovulation.

Of all sows that ovulated, 87 % showed vulvar reddening and 90 % showed boar estrus. Therefore, these characteristics were combined in order to determine whether

the prediction of ovulation could be improved. At onset of boar estrus, three categories of sows were defined on the basis of vulvar reddening (Table 3): sows that ceased to show vulvar reddening before onset of boar estrus (31 % of sows with both boar estrus and vulvar reddening), sows that did not show vulvar reddening until after the onset of boar estrus (13 %) and sows with a score red at the onset of boar estrus (56 %). The latter two categories of sows showed less variation in the timing of ovulation

Table 2. Variation (SD) in time between onset of an estrous phase and ovulation, before and after correction for the length of intervals between different estrous phases. A phase of estrus was defined as the period during which a standing response was exhibited to either a back pressure test (man estrus), presence of a boar (spont estrus), back pressure test (BPT) in presence of a boar (boar estrus), or BPT in presence of four boars (DMA estrus).

Ovulation, hours from	Related to duration of	n	Mean, h	SD1[a]	SD2[b]	β[b]	R^2
Onset of Man–estrus	$Int_{DMA\text{-}man}$	36	23	15	10	-0.6*	0.5
	$Int_{Boar\text{-}man}$	49	25	13	9	-0.5*	0.5
	$Int_{Spont\text{-}man}$	33	25	15	13	-0.4*	0.3
Onset of Spontaneous estrus	$Int_{Spont\text{-}man}$	33	26	17	13	0.6*	0.5
	$Int_{Boar\text{-}spont}$	59	23	15	12	-0.8*	0.3
	$Int_{DMA\text{-}spont}$	47	24	14	10	-0.7*	0.4
Onset of Boar estrus	$Int_{DMA\text{-}boar}$	76	35	13	10	-0.7*	0.4
	$Int_{Boar\text{-}man}$	49	36	13	9	0.5*	0.4
	$Int_{Boar\text{-}spont}$	59	38	13	13	0.2	0
Onset of DMA estrus	$Int_{DMA\text{-}boar}$	76	42	11	10	0.3*	0.1
	$Int_{DMA\text{-}man}$	36	44	12	10	0.4*	0.3
	$Int_{DMA\text{-}spont}$	47	43	11	10	0.3*	0.1

[a] SD1: Standard deviation of the uncorrected means, calculated for the sows in which the length of the concerning interval could be calculated. SD2: Standard deviation for the same sows (Mean Square Error), after correction for the length of the concerning interval (see model below). [b] β and R^2 calculated from the model: $Y_{ij} = \mu + \beta.Int_i + e_{ij}$; with Y_{ij} = h to ovulation and β = regression coefficient for length of interval Int_i. *$P < .05$

as expressed in hours after end of vulvar reddening compared with other sows. Ovulation took place within 36 h after and 2 h before the end of vulvar reddening in these sows. In contrast, sows that ceased to show reddening before onset of estrus ovulated within a range twice as large (70 h after to 5 h before end vulvar reddening).

Table 3. Onset of boar estrus[a] and end of vulvar reddening[b] in hours to ovulation, depending on the manifestation of vulvar reddening as related to the onset of boar estrus.

Time of ovulation, h	Category of sows	Time of ovulation, h				
		n	Mean, h	SD, h	Minimum	Maximum
From onset of boar estrus	All sows showing boar estrus	99	34	13	0	64
	Vulvar reddening ceased before onset of boar estrus	28	26	14	4	64
	Vulvar reddening at onset of Boar estrus	51	38	10	18	60
	No vulvar reddening until after Onset of boar estrus	20	38	11	14	56
From end of vulvar reddening	Vulvar reddening ceased before onset of boar estrus	28	30	19	-5	70
	Vulvar reddening at onset of boar estrus	51	18	10	-2	36
	No vulvar reddening until after onset of boar estrus	12	16	10	-1	33

[a] Boar estrus: phase of estrus during which standing response was shown to a back pressure test in the presence of a teaser boar. [b] End of vulvar reddening: 4 h after the last time inner vulva mucosa was judged as being red.

Discussion

This study is the first to relate different phases of behavioral estrus, monitored by applying different levels of stimuli during detection of estrus, to the timing of ovulation. The responsiveness to boar stimuli, used for detection of estrus, increased

in the period before ovulation. As a consequence, onset of DMA estrus occurred earliest relative to ovulation, followed by boar estrus. Onset of man estrus and spontaneous estrus, did not occur in the same order in all sows. Apparently the presence of a teaser boar (without BPT) and the back pressure test in absence of a boar are perceived differently by individual sows with respect to their potential to evoke a standing response.

To satisfactorily predict ovulation for the majority of sows, the predicted timing of ovulation should be within a 24-h range, which is the duration of the period of optimal fertilization (Soede et al., 1995). To achieve such a prediction with 90 % of the sows within this 24 h-range, the standard deviation of the predicted timing of ovulation must not exceed 7.5 h. Moreover, a predictor of ovulation is only suitable if it can be obtained in the majority of sows. Onset of estrus did not yield a prediction of ovulation with a standard deviation of less than 7.5 h at any of the stimulus levels used in this study. The least variation was found in the timing of ovulation relative to onset of DMA estrus (SD = 12 h). The range between the first sow and the last sow ovulating after onset of estrus was between 48 h (man estrus) and 64 h (boar estrus). Several studies reviewed by Soede and Kemp (1997) have shown the huge variation in the timing of ovulation after onset of boar estrus. In those studies, ovulation occurred 35 to 45 h after onset of estrus on average, and the range in the timing of ovulation mounted up to 70 h within a study. From the current study, it appears that at other stimulus levels, the range in the timing of ovulation relative to the onset of estrus is also large.

The large variation in timing of ovulation relative to onset of the different phases of estrus was related to the variation in the length of the intervals between phases. Length of these intervals explained 10 to 50 % of the variation in the timing of ovulation. Willemse and Boender (1967) suggested that in gilts, the duration of estrus as detected in absence of a boar (man estrus) always covers the middle two thirds of the duration of estrus as detected in presence of a boar (boar estrus); and that ovulation occurs during the second half of man estrus. This would imply that length of the intervals between phases of estrus is related to the time from onset of a phase to ovulation. From our data it appears that indeed there is a relation between the length of the intervals and the timing of ovulation. However, length of the intervals never

explained more than 50 % of the variation in the timing of ovulation, and the standard deviation in the predicted timing of ovulation never became lower than 9 h. Moreover, an increase in length of an interval was not always related to an increase in the interval from the onset of estrus to ovulation. For example, a 1-h increase in the interval from onset of boar estrus to man estrus was related to 0.5 h delay in the timing of ovulation relative to the onset of boar estrus, but 0.5 h decrease in the interval from onset of man estrus to ovulation. This is contradictory to the findings of Willemse and Boender (1967). The different stages of estrus could not be calculated for many sows because they did not show estrus at all stimulus levels. DMA estrus and boar estrus were recorded in 97 and 90 % of the sows, but man estrus and spontaneous estrus were recorded in only 46 and 56 % of the sows. The percentage of sows showing man and spontaneous estrus was particularly low in sows that were also submitted to the DMA-stimulus (41 and 54 % vs 68 and 68 % in sows without DMA stimulus). During the days preceding onset of estrus, these sows experienced extra boar contact, probably causing habituation to boar stimuli during detection of estrus later on (Langendijk et al., 2000). Low expression of estrus at a stimulus level reduces the applicability as a predictor of ovulation. In this study, a reasonable amount of variation (40 %) in the timing of ovulation relative to the onset of boar estrus could be explained by the interval from onset of DMA estrus to the onset of boar estrus in 76 sows. The difference between our findings and the concept of Willemse and Boender (1967) might be explained partly by the fact that their studies were with gilts. Besides, in their protocol for detection of estrus, detection was performed without a boar as soon as a standing response could be evoked in absence of a boar. As pointed out before, the high level of boar contact which was maintained throughout estrus in our protocol, might have affected expression of estrus and thereby the relation between duration of different phases of estrus and the timing of ovulation. With a protocol comparable to the current study, Soede et al. (1996) also found a weak relationship between duration of estrus detected in absence of the boar and duration of estrus detected in presence of the boar (R^2 = 0.25). Apparently, recording the increase in responsiveness in the course of estrus by applying different detection stimuli does not substantially improve the prediction of the timing of ovulation.

Relating the timing of ovulation to changes in vulvar reddening yielded the greatest error in the estimated timing of ovulation; ovulation took place from 70 h after to 5 h before the end of vulvar reddening. Vulvar reddening and vulvar swelling as a sign of approaching estrus are probably related to the rise in circulating estrogens during the follicular phase, which stimulate blood flow in the genital organs (Rojkittikhun et al., 1992). The density of estrogen receptors in pig uterine and cervical tissue (Stanchev et al., 1984; Stanchev et al., 1990) and in chimpanzee sex skin (Ozasa and Gould, 1982) increases enormously during the late follicular phase and is highest during estrus. Following the rise of the preovulatory LH surge, plasma estradiol concentrations start to drop and reach basal levels during the day before ovulation (van de Wiel et al., 1981). A concomitant decrease in related vulvar reddening might be expected. In our study, no vulvar reddening was scored after ovulation, except for five sows which ceased to show vulvar reddening within 5 h after ovulation. The end of vulvar reddening took place at 21 h before ovulation. These facts indicate that there is a relationship between the decline in circulating estrogens before ovulation and vulvar reddening, although the end of vulvar reddening ranged from 70 h before to 5 h after ovulation. The great range in the interval from the end of vulvar reddening to ovulation was related to the duration of vulvar reddening. In sows with a shorter duration of vulvar reddening, ovulation took place within a 75-h range from the end of reddening. In the sows with a longer duration of vulvar reddening the range in timing of ovulation relative to the end of vulvar reddening to ovulation was less, around 30 h.

Combining information on boar estrus and vulvar reddening gave a more precise estimation of the timing of ovulaton. Sows that showed vulvar reddening at or after onset of boar estrus (56 and 13 % of the sows that showed boar estrus) ovulated 17 h (SD = 10 h) after the end of vulvar reddening. End of vulvar reddening occurred within a range of 36 h before and 2 h after ovulation. In sows that had already ceased to show vulvar reddening at onset of estrus, this range was twice as large. This means that sows with vulvar reddening at onset of boar estrus, will hardly ever ovulate before the end of vulvar reddening. Insemination in these sows can be postponed until the end of vulvar reddening.

Implications

Onset of estrus as detected at different stimulus levels shows too much variation to be used as a predictor for the timing of ovulation. Recording the increase of responsiveness to boar stimuli used for detection of estrus explains some of this variation, but not enough to accurately predict ovulation. Moreover, not all sows show estrus at every stimulus level. The timing of onset and end of vulvar reddening relative to ovulation are too variable to predict ovulation. Vulvar reddening, however, hardly ever takes place after ovulation and is therefore a sign that ovulation still has to take place. This information can be used to postpone the first insemination in sows that still show vulvar reddening. Besides, sows that show vulvar reddening at or after onset of boar estrus, ovulate within a reasonable range from the end of reddening. Combination of information on vulvar reddening and onset of boar estrus can thus be used to correctly time insemination in about two thirds of sows.

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CHAPTER 3

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Effect of boar contact on follicular development and on estrus expression after weaning in primiparous sows

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ABSTRACT

Boar contact can induce ovarian activity, advance estrus and stimulate estrous behavior in sows. High amounts of boar contact can, however, suppress estrous behaviour. The present study with primiparous sows was designed to compare sows that had contact with a teaser boar during detection of estrus, with sows that had no boar contact at all. Number of sows detected in estrus within 9 d after weaning, onset and duration of estrus, follicular dynamics and timing of ovulation were studied. Boar contact increased the number of sows that ovulated and showed estrus from 14 of 47 to 24 of 47 ($P < 0.05$). Average timing of ovulation was later for sows with boar contact (165 h vs 150 h after weaning). Duration of estrus, detected without a boar, was similar in the two groups. For the sows with boar contact, duration of estrus detected with a boar was longer than estrus detected without a boar (56 vs 38 h; $P < .01$). Follicular dynamics were not affected by boar contact; boar contact only increased the number of sows with ovulation. Ovulatory sows showed a larger increase in follicular diameter ($P < 0.01$) from weaning to Day 4 after weaning (from 2.3 to 5.4 mm) than anovulatory sows (from 2.5 to 4 mm). Anovulatory sows did not show follicular growth after Day 4. It is concluded that boar contact can increase the number of sows that ovulate and show estrus after weaning. Estrous behavior does not seem to be suppressed by contact with a teaser boar, compared to sows without boar contact.

Key words: sows, boar, estrus, follicular

Introduction

After weaning, boar contact can induce the onset of ovarian activity and advance estrus in sows by days (8). Boar stimuli are probably important in stimulating neuro-endocrine pathways that are involved in the regulation of ovarian activity. Introduction to a boar has been shown to elicit LH release in sows that had been anestrous for 21 d (13). The effects of boar contact on follicular dynamics, however, have not been described previously. Besides inducing ovarian activity, boar stimuli can also affect expression of estrous behavior. Generally, boar presence stimulates estrous behavior, but excessive boar stimulation can reduce the expression of estrous behavior (4). In a study with sows (6), contact with a teaser boar three times a day for 5 min maximized

the number of sows detected in estrus after weaning. Extra boar stimulation did not increase the number of sows detected in estrus, nor the recorded duration of estrus. The extra boar stimulation reduced the number of sows exhibiting estrus in absence of a boar and reduced the duration of estrus in presence of the teaser boar. It was concluded from that study that an increased amount of boar contact reduces responsiveness to lower levels of boar stimuli, and that an increased amount of boar contact does not improve detection of estrus. Whether this also applies at lower levels of boar contact remains unclear. The present experiment was designed to study whether head-to-head contact with a boar during detection of estrus (three times a day), compared to a situation with no boar contact at all, affects follicular growth, return to estrus and expression of estrus after weaning.

Materials and Methods

Animals and Housing

Ninety-four primiparous sows of a commercial breed (Landrace x Yorkshire crossbred) were brought to the experimental farm on the day of weaning (Day 0), every 2 w in batches of approximately 20 sows. After an average lactational period of 25 d (SD 3.4 d), average litter size was 9.8 (SD 1.3), the sows weighed 143 kg (SD 15.4 kg) on average, had lost 25 kg (SD 10 kg) on average, and had 12.8 mm (SD 2.6 mm) backfat. During lactation, sows were fed a liquid diet according to guidelines of a commercial feeding company (calculated average nutrient intake: 790 g CP/d, 65.5 MJ ME/d; ME = metabolizable energy). After weaning, sows received a pelleted feed, and were allowed a feed intake twice the maintenance level (ME_m = 440 $kJ.BW^{.75}.d^{-1}$), but consumed 85% (SD 19%) of their feed allowance on average from day 0 to 5. The sows were housed in individual crates and fed twice daily, at 0830 and 1630.

Treatments

At weaning, sows were assigned to a 2 x 2 factorial design, distributing body weight and back fat thickness equally among treatments. The two factors were 1) boar

contact during detection of estrus (yes/no), and 2) the diet fed from weaning until ovulation. Half of the sows were fed a starch-rich diet, the other half a fat rich diet. The most relevant effects of the diets are presented in this paper; further details on the diets and effects of the diets on reproductive performance are discussed extensively by van den Brand et al. (12). Half of the sows received boar contact during detection of estrus. The other half did not receive any boar contact at all. Sows without boar contact were housed separately from the other sows, in a similar barn. To avoid confounding of the effect of boar contact with barn effects, treatments were allotted to a different barn every batch.

Detection of Estrus

Sows in the treatment with boar contact were first confronted with a boar on Day 2, once for approximately 10 min, and thereafter only during detection of estrus. The boar was housed in a separate barn. Detection of estrus was performed daily at 0800, 1600 and 2400, starting at 1600 on Day 3, and ending at 1600 on Day 8. Detection of estrus was performed by four experienced staff members, one person performing the detection for both treatments at a given time. First, sows in both treatments were checked for a standing response in absence of a boar, by rubbing the sows' flank and pressing its back (back pressure test, BPT). Sows were deemed to be in estrus when they responded to the BPT with a frozen stance, arched back, and cocked ears (standing response). Second, only the sows with boar contact were checked for standing response in the presence of a teaser boar. The teaser boar was led in front of four to five sows at a time, and was allowed to have head-to-head contact with these sows. After approximately 1 min, a BPT was performed to test for standing response. In total, the boar remained in front of the five sows for approximately 5 minutes. Onset and end of estrus were defined as 4 h before the first time a standing response was shown and 4 h after the last time a standing response was shown.

Ultrasonography

Transcutaneous ultrasonography with a 5 Mhz annular array sector probe (Pie Medical, Maastricht, The Netherlands) was performed on Days 0, 4, 6 and 8 for all sows to

monitor follicular development. Maximum follicle size was used for analyses. Follicle size of the largest follicle was regarded as indicative of follicular growth. On sows in estrus and on sows with follicles larger than 5 mm, ultrasound was performed every 8 h, to record timing of ovulation and silent ovulation. Timing of ovulation was defined as the average of the last time Graafian follicles were seen and the first time they were absent on the ovary. Ovulatory follicle size was defined as the size of the follicles at the last scan before ovulation. On Day 8 all sows were monitored again to check for silent ovulation and for cystic follicles. None of the sows experienced lactational estrus, as could be concluded from the absence of corpora lutea on Day 0.

Statistical Analyses

Data were analyzed in SAS (9). Data on the interval from weaning to estrus and ovulation, on the duration of estrus, and on the timing of ovulation relative to the period of estrus were analyzed using a general linear model: $Y_{ij} = \mu + B_i + D_j + B_i{*}D_j + e_{ij}$ with B_i boar contact (yes/no) and D_j the diet fed after weaning (starch/fat). To test the effect of boar contact, only characteristics of estrus detected in absence of the teaser boar were analyzed. For analysis of the duration of estrus, the interval from weaning to estrus was included as a covariate. The difference in duration of estrus between estrus detected in presence of a boar and estrus detected in absence of a boar was tested using the paired T-test, for sows in the treatment with boar contact. Data on the number of sows ovulating and showing estrus in each treatment (B_i) were analyzed using the χ^2 test for a 2 x 2 table. Data on follicular development were analysed with a nested general linear model, regarding the diameter on the different days (Days 0, 4, and 6) as repeated measurements within one sow: $Y_{ijkl} = \mu + O_i + B_j + D_k + Sow(O_i * B_j * D_k) + Day_l + Day_l * O_i + e_{ijkl}$; with O_i = ovulation (yes/no), B_j = boar contact (yes/no), D_k = diet (fat/starch) , and Day_l = Day 0, 4 or 6. Main effects (O_i, B_j, and D_k) were tested against between-sow-effects. Interactions other than those mentioned in the model were not significant (for all characteristics) and therefore were omitted. Pearson's correlation coefficients were calculated for the relationships among follicle diameters on different days and estrus characteristics.

Results

Boar contact during detection of estrus increased the number of sows ovulating within 9 d after weaning ($P < 0.05$); of the 47 sows with boar contact 24 ovulated, whereas of the 47 sows without boar contact only 14 ovulated (Table 1). Average timing of ovulation and average onset of estrus were later in sows with boar contact ($P < 0.05$). The majority of the extra sows that ovulated in the treatment with boar contact, ovulated relatively late compared to sows without boar contact (Figure 1). Boar contact did not affect expression of estrus in the sows with ovulation. The percentage of sows exhibiting estrus in absence of the teaser boar (86% vs 92%) and the duration of estrus recorded in absence of the teaser boar (39 h vs 38 h), did not differ between the two treatments (Table 1). For the treatment with boar contact, onset of estrus was detected earlier (128 h vs 137 h; $P < 0.01$) and duration of estrus was longer (56 vs 38 h; $P < 0.01$) when recorded in presence of the teaser boar, compared to detection in absence of the boar (Table 1).

Diet did not affect the number of sows ovulating (18 vs 20). However, the fat-rich diet delayed the onset of estrus (146 h vs 116 h after weaning; $P < 0.01$) and delayed ovulation (170 h vs 150 h after weaning; $P < 0.01$). There was no interaction between boar contact and diet for any of the estrous characteristics.

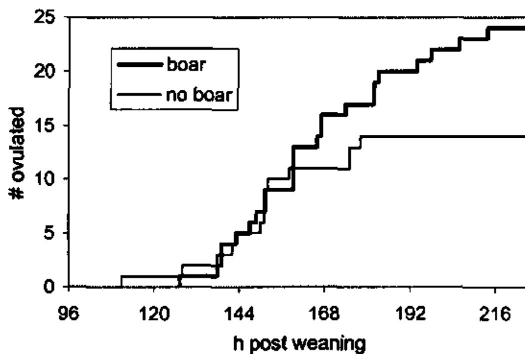

Figure 1. Effect of boar contact on cumulative number of sows ovulating in each treatment in relation to time from weaning. Forty-seven sows were assigned to each treatment (boar contact/no boar contact).

Table 1. Expression of estrus in presence (BPT+boar) or absence (BPT) of a teaser boar, and timing of ovulation for sows with boar contact and sows without boar contact during detection of estrus[a].

Detection		No boar contact		Boar contact	
	Number of sows	47		47	
	Number of sows ovulating[a] (%)	14[c]	(30)	24[d]	(51)
	Weaning to Ovulation in h (SD)	150[c]	(18)	165[d]	(23)
BPT	Number of sows detected (%)	12	(86)	22	(92)
	Weaning to Estrus in h (SD)	119[c]	(23)	137[d]	(29)
	Onset Estrus – Ovulation in h (SD)	29	(11)	26	(13)
	Duration of estrus in h (SD)	39	(12)	38	(15)
	Ovulation as % of estrus (SD)[b]	74	(17)	73	(38)
BPT + Boar	Number of sows detected (%)			24	(100)
	Weaning to Estrus in h (SD)			128	(26)
	Estrus to Ovulation in h (SD)			37	(7)
	Duration of estrus in h (SD)			56	(9)
	Ovulation as % of estrus (SD)			67	(11)

[a]Estrus or ovulation within 9 d after weaning. Detection of estrus was first performed in absence of a boar for all sows, and second, for sows with boar contact, in the presence of a teaser boar.
[b]Ovulation as % of estrus was calculated as the ratio between the time from onset of estrus to ovulation and the duration of estrus. [c,d] Different superscripts indicate significant differences ($P < 0.05$) between columns, corrected for diet effect.

Follicle size on Days 0, 4, and 6, and ovulatory follicle size were not affected by boar contact or diet (Table 2). Boar contact only increased the number of sows that ovulated within 9 d (ovulatory sows). Anovulatory sows had a lower average body weight (140 kg vs 147 kg; $P < 0.05$) and lower backfat thickness (12 mm vs 14 mm; $P < 0.05$) at weaning. For ovulatory sows, follicle size increased from Day 0 to Day 4 ($P < 0.01$), and from Day 4 to Day 6 ($P < 0.01$). For anovulatory sows, follicle size increased from Day 0 to Day 4 ($P < 0.01$), but not thereafter. Ovulatory sows had larger follicles on Day 4 and Day 6 than anovulatory sows ($P < 0.01$), although the range of follicle size among sows was large. On Day 4, follicle size ranged from 2 to 6

mm in the anovulatory group and from 3 to 7 mm in the ovulatory group. On Day 6, follicle size ranged from 2.5 to 7 mm in anovulatory sows and from 4 to 8 mm in ovulatory sows. On Day 0 follicle size did not differ between ovulatory and nonovulatory sows. However, follicle size on Day 0 was correlated with follicle size on Day 4 for all sows (r = 0.20; $P = 0.06$; n = 88). For sows that ovulated, follicle size on Day 4 was correlated with the interval from weaning to ovulation (r = -0.29; $P = 0.08$; n = 36) and with the interval from weaning to estrus, recorded in the absence of a boar (r = -0.40; $P = 0.02$; n = 32), or in the presence of a boar (r = -0.68; $P < 0.01$; n = 23). Follicle size on Day 0 was not related to any of these characteristics.

Discussion

In primiparous sows, boar contact during estrus detection seems essential to induce the onset of ovarian activity and behavioral estrus for a proportion of sows. In this study, boar contact increased the number of sows ovulating within 9 d from 14 of 47 to 24 of 47 ($P < 0.05$). Most of the extra ovulating sows in the group with boar contact ovulated later compared to the sows without boar contact. As a consequence, average ovulation and average onset of estrus were later for the sows with boar contact. Boar contact did not affect follicular dynamics, but increased the number of ovulatory sows. Follicular dynamics indicated whether sows would ovulate or not. Initial follicle growth (Day 0 to 4) was greater for ovulatory sows. After Day 4 anovulatory sows did not show an increase in average follidular size. Lucy et al. (7) also found reduced follicular growth in anovulatory sows from Day 0 to 4, with failure to achieve ovulatory size. Anovulatory sows do show some initial follicular growth after weaning, even up to 6 mm on Day 4. However, this does not result in ovulatory-size-follicles.

The effect of boar contact on the number of ovulatory sows is probably mediated by an increase in pituitary activity. Olfactory elements of boar presence, for example, might cause the central nervous system to release hormones and neuropeptides that are involved in the regulation of LH pulsatility (1). Introduction to a boar increased LH pulsatility in sows that had been anestrous for 21 d after weaning (13). Kingsbury and

Table 2. Effect of boar contact on maximum follicle size (Means and SD) on Day 0 (weaning), 4, 6 and on Day 8 or Day of ovulation, for ovulatory and anovulatory sows.

		D0			D4			D6			D8/ovulation[c]		
		N	Ø (mm)	SD (mm)	N	Ø (mm)	SD (mm)	N	Ø (mm)	SD (mm)	N	Ø (mm)	SD (mm)
OVU[a]	B[b]	22	2.3^{f}	0.7	23	$5.3^{d,g}$	1.1	19	$6.7^{d,h}$	1.0	24	7.2	0.8
	NB	14	2.4^{f}	1.2	13	$5.5^{d,g}$	1.0	11	$6.4^{d,h}$	1.1	14	6.6	1.2
ANOVU	B	23	2.2^{f}	0.9	23	$3.7^{e,g}$	1.4	19	$4.3^{e,g}$	1.2	23	3.9	1.2
	NB	32	2.7^{f}	0.8	32	$4.2^{e,g}$	1.0	31	$4.2^{e,g}$	1.0	32	3.8	1.0

[a] OVU: ovulatory sows, ANOVU: anovulatory sows.
[b] B: boar contact, NB: no boar contact.
[c] For ovulatory sows follicle size at ovulation is given.
[d,e] Different superscripts indicate significant differences ($P < 0.05$) between rows.
[f,g,h] Different superscripts indicate significant differences between columns.

Rawlings (5) found that LH pulsatility increased immediately after first exposure to a boar, in gilts that showed estrus within 2 mo after this boar exposure. In gilts that did not show estrus withinthose 2 mo and in control gilts (no boar exposure), the increase in LH pulsatility after boar exposure was not observed. Follicle growth in later stages of the follicular phase is dependent on LH pulsatility (2,3). Boar contact might have increased LH pulsatility in the present study to a level sufficient to stimulate follicular growth toward ovulation, in sows that otherwise would not have ovulated and shown estrus. Anovulatory sows that showed initial follicular growth were probably insensitive to the boar stimuli or not able to react adequately, probably because of metabolic restraints. This was indicated by a lower average body weight (140 kg vs 147 kg; $P < 0.05$) and lower backfat thickness (12 mm vs 14 mm; $P < 0.05$) at weaning, for the anovulatory sows. Primiparous sows are known to have a poor body condition at weaning, resulting in a relatively high incidence of delayed estrus or anestrus (14). In primiparous sows delayed estrus is related to low LH pulsatility on the day of weaning (11). In the present study, ovulatory sows had better body condition, probably rendering them more sensitive to boar stimuli. These stimuli might have increased LH pulsatility sufficiently to result in ovulation in these sows.

For treatment with boar contact, duration of estrus in the presence of a boar was longer (56 h) than duration of estrus recorded in treatment without boar contact (39 h). Moreover, treatment with boar contact resulted in detection of estrus in 24 of the 24 ovulating sows, whereas treatment without boar contact resulted in detection of estrus in 12 of the 14 ovulating sows (NS). In an earlier study (6), we performed estrus detection consecutively in the absence of a boar, in the presence of a boar and, for some sows, additionally in the presence of four boars simultaneously. Contact with four boars during detection of estrus did not improve the detection of estrus. The number of sows detected in estrus and the duration of estrus were the same as recorded in presence of the teaser boar in sows that were not confronted with the four boars. It thus seems that detection of estrus is optimal when performed with a teaser boar. More boar contact during detection does not necessarily improve detection (6), whereas no boar contact during detection decreases detection (this study). From the same study (6), we also found that for sows that were confronted with four boars during detection of estrus, expression of estrus was reduced at the lower levels of

detection stimuli. Responsiveness to detection stimuli was lower for sows that were confonted with four boars, compared to the sows that were only confronted with a teaser boar. In the present study two lower levels of boar contact were compared. Expression of estrus in absence of a boar was similar for both levels of boar contact; sows that received boar contact responded similarly to the back pressure test in absence of the boar as the other sows. Duration of estrus (38 vs 39 h) and the percentage of sows exhibiting estrus (86 vs 92%) in the absence of a boar were equal for treatments with and without boar contact. Limiting boar contact during detection of estrus to the use of a teaser boar, therefore, seems to optimise detection of estrus without reducing responsiveness to detection stimuli.

Implications

Boar contact can affect follicular growth in primiparous sows, increasing the number of sows ovulating and showing estrus. Limiting boar contact to the use of a teaser boar during detection of estrus seems to result in optimal detection of estrus.

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CHAPTER 4

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Myometrial activity around estrus in sows: Spontaneous activity and effects of estrogens, cloprostenol, seminal plasma and clenbuterol

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ABSTRACT

A new, non-surgical, open-end catheter technique was used to study spontaneous uterine activity around estrus in sows, and to study effects of estrogens, seminal plasma, cloprostenol, and clenbuterol. In the first experiment, uterine activity was studied in 14 multiparous, cyclic sows, during 1 or more estrous cycles, from Day –4 to Day 4 of the cycle (Day 0: first day of standing estrus). From a few days before estrus until estrus, the percentage of sows showing any uterine contractions increased from 55 % to 100 %, and frequency and mean amplitude of uterine contractions for these sows increased from 15 h^{-1} to 22 h^{-1}, and from 20 mmHg to 40 mmHg on average. After estrus, uterine activity decreased again. There were large differences between sows in uterine activity, which were consistent over the days of the cycle. In the second experiment, 11.5 µg of estrogens in 100 ml saline (n = 17), 100 ml seminal plasma (n = 5), 1 mg cloprostenol in 100 ml saline (n = 10), 0.30 mg clenbuterol in 100 ml saline (n = 11), or 100 ml saline (n = 5) was infused iu, after recording spontaneous activity. Infusion with saline or seminal plasma did not affect uterine activity. Estrogens increased frequency of contractions. Cloprostenol increased both frequency and amplitude of contractions. Clenbuterol reduced both frequency and amplitude of contractions. In conclusion, this study shows that spontaneous uterine activity in sows is increased around estrus, and supports the role of estrogens in boar seminal plasma in affecting uterine activity around mating. Furthermore, this study has yielded possible tools to study the relation between uterine activity and sperm transport.

Key words: sows, uterine activity, estrus, estrogens, prostaglandin, seminal plasma, clenbuterol

Introduction

In swine, myometrial activity during estrus is supposed to play a role in transport and distribution of semen through the female genital tract (Zerobin & Sporri, 1972). The activity of the myometrium is myogenic in origin, and is driven by pace-maker activity of the uterine smooth muscle cells. In several species, the myometrium has been shown to be subject to endocrine changes throughout the estrous cycle (Rodriguez-Martinez et al., 1987; Devedeux et al., 1993). Besides cyclic changes in myometrial

activity, exogenous factors can affect myometrial activity. For example, estrogens in boar seminal plasma have been shown to trigger release of prostaglandin from the endometrium, and to stimulate myometrial activity (Claus, 1990). Such factors might therefore be of importance in affecting semen transport and subsequent fertilization.

In the sow, there is little quantitative information on the change in myometrial activity around estrus, on the variation between sows in myometrial activity. Available information is based on studies with a small number of sows, using surgical techniques to record electrical activity (Scheerboom et al., 1987; Claus et al., 1989) and stretch (Bower, 1974). In terms of sperm transport, intraluminal pressure variation might be a better indicator of uterine contractions, but this has so far only been reported by Docke and Worch (1963). In the present study, two experiments are described in which a new, non-surgical technique was used to record intraluminal pressure. With this technique, it is possible to study myometrial activity in a relatively large number of sows, in a relatively undisturbing situation. In the first experiment, spontaneous myometrial activity was recorded everyday throughout the period around estrus, to study the variation between sows in the change in uterine activity. In the second experiment, several exogenous factors were studied. The effects of uterine infusion with seminal plasma and a physiological amount of estrogens were investigated. Furthermore, effects of uterine infusion with cloprostenol (a prostaglandin analogue) and clenbuterol were studied, as a possible agonist and antagonist of myometrial activity in future studies.

Materials and Methods

Animals and Housing

Two experiments were performed, the first to study spontaneous myometrial activity around estrus, and the second to study the effect of several different uterine infusions. Both experiments were approved by the Ethical Committee for animal experiments of the Wageningen University. Multiparous, cyclic sows of a commercial breed (Yorkshire x Dutch Landrace) were used in both experiments. Sows were housed individually in

crates (2.2 m x 0.65 m) and were fed twice daily (0730 and 1700) with a commercial diet (2.5 kg d^{-1}, 12.9 MJ ME kg^{-1} and 130 g CP kg^{-1}). Detection of estrus was performed twice daily (after feeding) with a teaser boar. A sow was regarded in estrus when showing a standing response in reaction to manual back pressure in presence of a boar. Measurement of uterine activity took place between 0900 and 1700.

Measurement of Uterine Activity

Measurement of uterine intraluminal pressure changes was performed by adapting a device used for non-surgical embryo tranfer in pigs (Hazeleger & Kemp, 1994). The device consists of a flexible PVC rod (80 cm long; outer diameter 3 mm), with a 90° bend at the end, 1 cm in length. A Teflon guiding tube (length 1.2 m; outer diameter 2.4 mm), attached to the rod, serves to pass the open end measuring catheter (outer diameter 1 mm) into the uterine lumen. A second guiding tube was attached to the rod in experiment 2, to allow intra-uterine infusions. The PVC-rod enables to pass through the cervix, into the caudal part of the uterine horns. With this technique, it is possible to perform measurements on one sow repeatedly, during one cycle and over different cycles, without having to perform surgery or to apply anesthesia. For each measurement, the device is inserted and taken out after termination of the measurement. Before inserting the device, the vaginal area was thoroughly washed and desinfected with a 1:20 solution of Hibitane® (Zeneca BV, Ridderkerk, The Netherlands). The inserting device and the open end catheter were washed with alcohol, saline and Hibitane solution and then lubricated with an antiseptic gel (Instruvet, Amerongen, The Netherlands). With the unsedated sow in standing position, the device was maneuvered through the cervix, by rotating and pressing the PVC-rod along the cervical folds. A sudden decrease in resistance while maneuvering the device deeper into the genital tract was the sign for having entered the uterine lumen. Upon passage of the cervix, the device was gently pushed into the uterus, till 50 to 60 cm of the PVC-rod was inserted into the genital tract, i.e. approximately 20 to 30 cm into the caudal part of the uterus or one of the uterine horns. Care was taken to keep the measuring device in the same position throughout a measuring period, to avoid variation due to the position of the catheter tip. After inserting the device into the uterine lumen, the guiding tube was flushed with approximately 5 ml saline to

remove cervical mucus from the tip, and the fluid filled measuring catheter was passed through the guiding tube, into the uterine lumen. The measuring catheter was connected to a Statham (P23 Db) pressure transducer (Statham, Zoetermeer, The Netherlands), which was positioned at a fixed height, and connected to an amplifier (Godart-Statham, Zoetermeer, The Netherlands). The signal from the amplifier was filtered by a low pass filter with a cutoff at 0.23 Hz (kindly provided by J. Korterik, University of Twente, The Netherlands), and recorded on a pen recorder (Pharmacia, Woerden, The Netherlands) and a data-logger (Grant SQ1201, Catec, The Netherlands). From entering the uterine lumen until the start of recording, approximately 10 min elapsed. During measurement, the inserting device remained positioned in the uterus and was taken out, together with the open end catheter, after the measurement had terminated.

Correct insertion and functioning of the open end catheter was confirmed by the appearance of an oscillating baseline on the paper recorder (Fig. 1), caused by the respiratory movements of the sows body. The catheter was flushed with 1 ml saline, whenever the respiratory pressure fluctuations were no longer visible on the pen recorder, because of presumed obstruction of the catheter tip (1 to 5 times per h, on average). During measurement, notes were taken on the activity of the sow e.g. lying, standing, moving, tail moving. From preliminary work it appeared that movement of the sow, manipulation of the inserting device during measurement and periodical flushing of the open end catheter did not affect contractile activity. A contraction was defined as a temporary rise (minimum duration 8 sec) in luminal pressure, exceeding 12 mm Hg above baseline pressure. Two consecutive rises in luminal pressure were considered separate contractions, when the second rise occurred after pressure had decreased below 6 mmHg above baseline pressure, for at least ten seconds. These criteria were based on preliminary analyses, and were chosen to discriminate between contractile activity and artifacts in recorded pressure caused by e.g. movement of the sow. Amplitude of a contraction was the highest pressure recorded during the contraction, and expressed in mm Hg. Duration of a contraction was the time between the rise in pressure and the return to baseline pressure. Frequency of contractions was expressed in number per hour. Myometrial activity recorded during one measurement

(ca 1 h) was characterized by frequency of contractions, mean amplitude of contractions, and mean duration of contractions during the measurement.

Experiment 1

Fourteen sows were used to record spontaneous myometrial activity during and around estrus. Measurements were performed daily, during 45 to 60 min, starting 5 d before the onset of expected estrus. Onset of estrus was estimated on the basis of previous cycles. To avoid effects of estrus detection, measurements did not start until 90 min after estrus detection. Data were obtained on 29 estrous cycles from 14 sows, yielding 135 measurements of spontaneous myometrial activity during the follicular phase and the early luteal phase (Day –4 to Day 4; Day 0 is first day of estrus). For 7 of the 14 sows, measurements were performed during more than one cycle: for 3 sows during 4 cycles, for 2 sows during 3 cycles, and for 2 sows during 2 cycles. For practical reasons it was not possible to obtain measurements in consecutive cycles for all sows.

Data on frequency and amplitude of myometrial contractions were analyzed in SAS (SAS Inst. Inc., Cary, NC) with the GLM procedure using the following model $Y_{ij} = \mu + ED_i + C_j(ED_i) + D_k + e_{ijk}$ with ED_i the duration of estrus (short: 1.5 to 2.5 d, n = 13; long: 3 to 4 d, n = 15), C_j the number of the cycle and D_k (Day -4, .., Day 3) the day of the cycle. From some sows, too few data were obtained on consecutive cycles to account for a cycle-within-sow-effect in the model. Therefore, Cycles (Cj) were considered independent, and received a unique number (Cj), regardless of the fact that some cycles belonged to the same sow. All independent variables were included as class variables. Preliminary analysis showed that time of measurement (morning or afternoon), and interaction between cycle and day of the cycle number (C_j*D_k) did not affect myometrial activity. These were therefore excluded from the model.

Experiment 2

Twelve cyclic sows were used to determine effects of iu infusions (estrogens, cloprostenol, saline, seminal plasma or clenbuterol) on myometrial activity. Measurements were performed on Day 0 and Day 1 and 1 or 2 d before expected

estrus. After recording of spontaneous activity for 45 min, one of the following solutions was infused. 1. A mixture of Estrogens (Organon, Oss, The Netherlands) in 100 ml saline (0.9 % NaCl). The mixture consisted of 5 μg estradiol-17β, 2 μg estrone and 4.5 μg estrone-sulphate, being the same composition and amount as found in an average boar ejaculate (5). Stock solutions of estrogens were prepared by dissolving crystallized estrogens in 96 % alcohol and storing at 4 °C. Before infusion, alcohol was vaporized under continuous flow of N_2 gas, and 100 ml of saline was added. 2. Seminal plasma (100 ml). Seminal plasma was collected during October and November (Winter) from four different breeding boars (Dalland Breeding, Merselo, The Netherlands), centrifuged (3000 rpm during 15 min) to remove sperm cells and stored at –20 °C. At the end of the collection period, collected seminal plasma was thawed, pooled, and stored in 100 ml aliquots at –20 °C. Before infusion, the seminal plasma was warmed to room temperature. Estradiol-17β content in seminal plasma was analyzed by RIA at the Institute for Animal Science and Animal Health (Lelystad, The Netherlands), as described by van de Wiel et al. (32). The seminal plasma pool contained 80 pg/ml estradiol-17β. 3. Cloprostenol (Estrumate®, Mallinckrodt, The Netherlands): 1000 μg in 100 ml saline. The dosage for Cloprostenol was based on a pilot-study (unpublished results), in which 250 and 500 μg of Cloprostenol did not clearly affect intraluminal pressure variation. 4. Clenbuterol-HCl (Planipart ®, Boehringer-Ingelheim, Alkmaar, The Netherlands): 0.30 mg in 100 ml saline. The dosage for Clenbuterol was chosen from a pilot-study (unpublished results), in which 0.03 mg and 0.15 mg did not clearly affect intraluminal pressure variation. 5. Saline (100 ml). Infusion was performed through a second guiding tube, attached to the PVC-rod. After start of infusion of one of the substances, recording of uterine pressure went on for around 60 min.

Contractile activity before and after infusion was quantified as in experiment 1. Additionally, cumulative amplitude was calculated as the sum of the amplitude of all contractions in a certain period, and served as a measure for 'total activity'. Effects of infusion were analyzed per measurement as the difference between contractility after infusion and before infusion, according to the following model in SAS: $Y_{ij} = \mu + A_i + b*F_j + e_{ij}$ with Y_{ij} the difference in contractility (frequency, amplitude, duration, or cumlative amplitude of contractions), A_i the infused substance and F_j the frequency of contractions before infusion. Preliminary analyses showed that day of the cycle (Day –

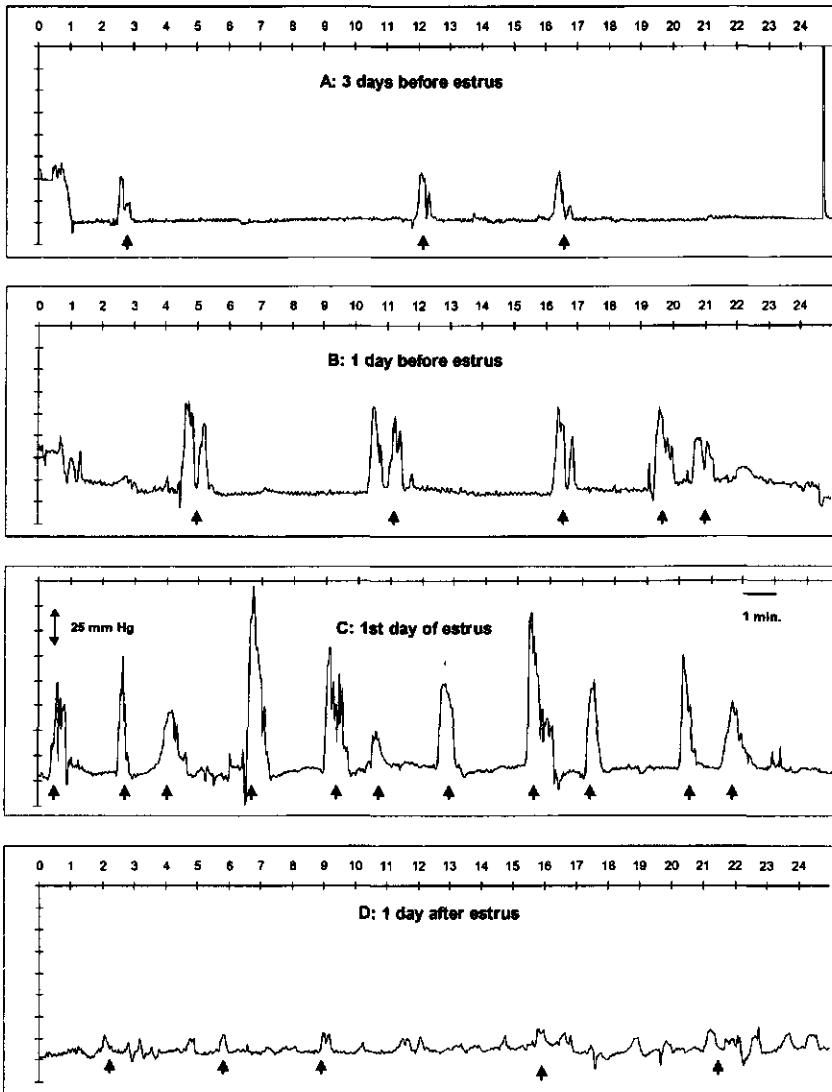

Figure 1. Spontaneous intra-uterine pressure variations for one sow on A: Day −3, B: Day −1, C: Day 0, and D: Day 3 (Day 0 is first day of standing estrus). Small variations in pressure in the periods between contractions reflect luminal pressure changes due to respiratory movements of the sow. Change in basal level at the start of panels A and B is caused by lying down of the sow. The sharp peak at 25 min in panel A is caused by flushing of the pressure sensor. Arrows indicate contractions, according to the applied definitions.

2, -1, 0 or 1) was not related to effect of infusion, and that contractility during 0 to 30 min after start of infusion did not differ from contractility during 30 to 60 min after infusion. These factors were therefore excluded from further analyses, and contractility after infusion was calculated over a 60 min period after start of infusion. The time between the start of an infusion and its effect on myometrial activity, was determined by plotting the cumulative amplitude in time. The slope in the increase in cumulative amplitude during the period before infusion and during the period after infusion was detemined manually. The time point where the increase in cumulative amplitude after infusion started to deviate from the increase before infusion was regarded as the onset of the effect of infusion.

Results

Experiment I

Figure 1 shows the intra-luminal pressure variation for one sow during several days around estrus. The small, frequent pressure fluctuations (2 to 4 mmHg) around the baseline are caused by respiratory body movements. Changes in luminal pressure superimposed upon the basal pressure always consisted of increase in pressure; decrease in pressure below baseline level was never observed. During the early follicular phase the myometrial activity started to increase. The percentage of measurements without any contraction during the 45 to 60 min of recording decreased from around 45 % a few days before estrus to 0 % during estrus ($P < 0.05$), and increased again after estrus (Fig. 2). For measurements with at least one contraction during the recording session, average frequency and amplitude of myometrial contractions increased ($P < 0.05$) from 15 h^{-1} and 20 mmHg a few days before estrus, to 22 h^{-1} and 40 mmHg on the first day of estrus (Fig. 2). Average frequency and amplitude of myometrial contractions declined again during the first days after estrus. The relative change in mean amplitude of contractions (approximately 100% increase from pro-estrus to estrus), was higher than the relative change in frequency of contractions (approximately 30% increase from pro-estrus to estrus).

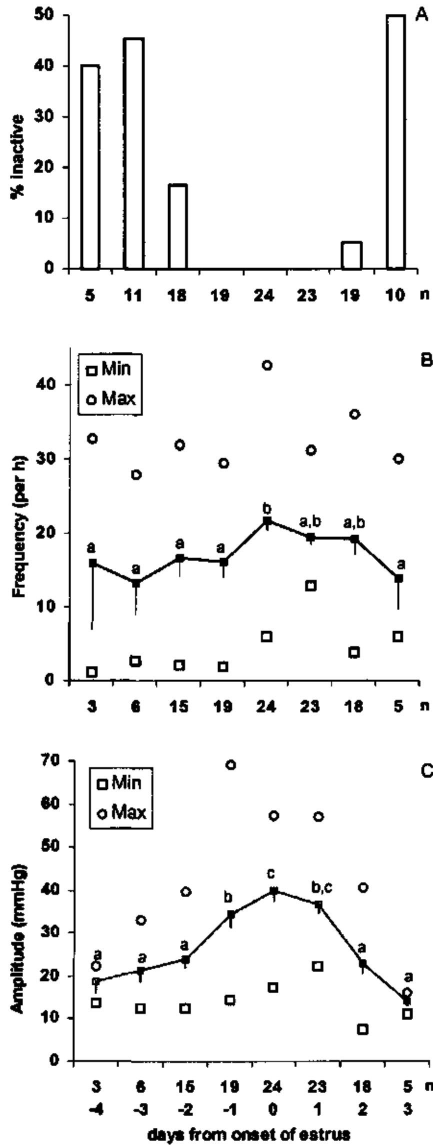

Figure 2. Average myometrial activity around estrus. Numbers below the X-axis indicate numbers of sows and days relative to the first day of standing estrus (Day 0). Minimum and maximum values are indicated by □ and O. A. Percentage of sows with a quiescent uterus, B. Frequency, and C. Amplitude of uterine contractions for sows with uterine activity. [a,b,c]Different superscripts indicate difference in frequency or amplitude between days of the cycle.

Variation in myometrial activity between different estrous cycles was large, for both frequency and amplitude of contractions ($P < 0.05$). Frequency of myometrial contractions ranged from 2 h^{-1} to 30 h^{-1} during the days before estrus, and from 6 h^{-1} to 40 h^{-1} during estrus. Mean amplitude of contractions ranged from 11 mmHg to 39 mmHg during the days before estrus, and from 16 mmHg to 57 mmHg during estrus. However, the difference between cycles in frequency and amplitude of contractions was consistent over the different days of the cycle; there was no interaction between Day of cycle (Di) and Cycle number (Cj). Duration of contractions did not change in the period around estrus, and remained around 60 sec per contraction on average. Duration of estrus (1.5 d to 2.5 d or > 2.5 d) did not affect frequency of contractions on any day of the cycle. In contrast, animals with a short estrus had a lower amplitude of contractions (19 vs 27 mmHg; $P < 0.05$) on Day 2. For animals with a short estrus, Day 2 was the first day post estrus; animals with a long estrus were still in estrus on Day 2.

Experiment 2

Frequency, amplitude and duration (not shown) of uterine contractions were not affected by infusion of 100 ml Saline, or by infusion of 100 ml Seminal plasma (Fig. 3A and 3B). Cloprostenol increased both frequency (from 11 to 22 h^{-1} ; $P < 0.01$), and amplitude (from 44 to 55 mmHg; $P < 0.05$) of uterine contractions. Estrogens increased frequency of contractions (from 12 to 17 h^{-1}; $P < 0.01$), but did not affect amplitude of contractions. Clenbuterol decreased both frequency (from 18 to 6 h^{-1}; $P < 0.01$) and amplitude (from 57 to 47 mmHg; $P < 0.05$) of uterine contractions. On average, the effect of infusion of Cloprostenol, Estrogens, and Clenbuterol was visible from around 10 min after start of infusion. The change in uterine motility, as characterized by the change in the slope of cumulative amplitude of contractions, occurred 11 min (5 to 20 min), 12 min (0 to 30 min) and 10 min (5 to 15 min) after start of infusion, respectively (Fig. 4A-C). Within the period of measurement (until 1 h after infusion), the effect of Cloprostenol, Estrogens, and Clenbuterol did not decrease.

Variation between cycles in frequency and amplitude of contractions was large. The rate of increase in cumulative number of contractions differed considerably between

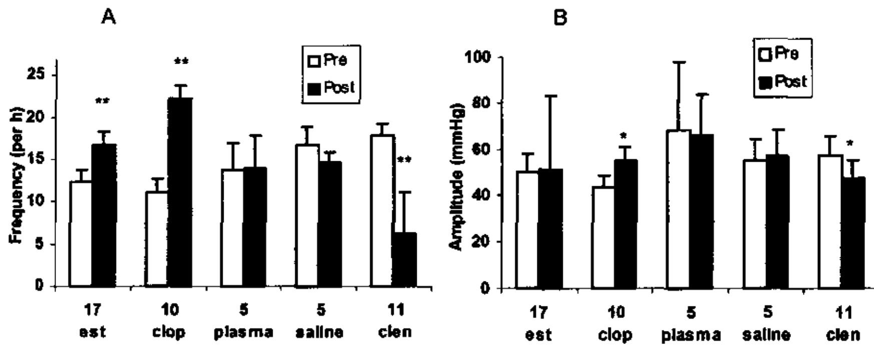

Figure 3. Frequency (A) and amplitude (B) of uterine contractions before and after iu infusion with 11.5 μg estrogens (est), 1000 μg cloprostenol (clop, a prostaglandin analogue), saline, seminal plasma (plasma), or 0.30 mg clenbuterol (clen), in 100 ml volumes. ** Indicates significant increase in frequency of uterine contractions ($P < .01$). * Indicates significant increase in amplitude of uterine contractions ($P < .05$).

measurements, both before and after the different infusions (Fig. 4). During 2 recordings, no uterine activity was observed before infusion. After infusion with Cloprostenol, uterine activity was triggered towards 1 and 6 contractions per h, with amplitude of 31 and 169 mmHg, respectively. The effect of infusates on frequency and mean amplitude of contractions was not consistent within treatments (Fig. 4 A-C). After infusion with Cloprostenol, frequency of contractions increased for all measurements, but amplitude was slightly decreased for 2 of the 12 measurements (-6 and -8 mmHg). After infusion with Estrogens, frequency did not change or slightly decreased (0 to –3 h^{-1} change) for 7 of the 17 measurements, and amplitude decreased for 6 of the 17 measurements (-2 to -28 mmHg). After infusion with Clenbuterol, frequency of contractions decreased for all measurements, but amplitude increased for 3 of the 11 measurements (+1 to + 51 mmHg). However, the cumulative amplitude per h, i.e. the sum of the amplitude of contractions in 1 h, did show a consistent change ($P < 0.05$) within these three treatments. For Cloprostenol and Estrogens, a positive change (+531 $mmHg.h^{-1}$ and +230 $mmHg.h^{-1}$), and for Clenbuterol, a negative change (-738 $mmHg.h^{-1}$) in the cumulative amplitude per h was recorded for all measurements (not shown). The change in frequency or amplitude after infusion was not related to the frequency or amplitude of myometrial contractions before infusion.

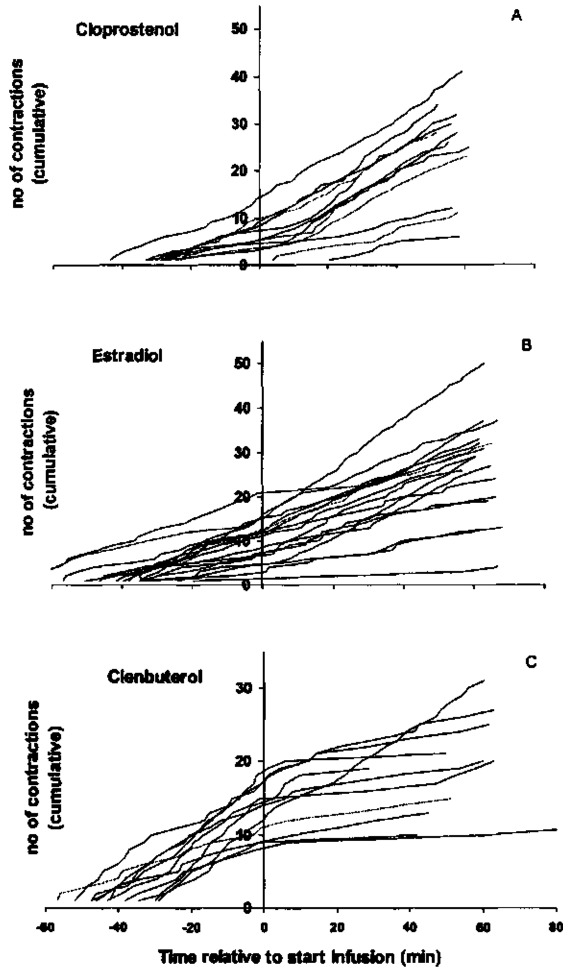

Figure 4. Cumulative increase in number of contractions for individual sows, relative to the start of iu infusion (t = 0). A. Infusion with a prostaglandin analogue (1000 μg cloprostenol). B. Infusion with estradiol (11.5 μg) C. Infusion with clenbuterol (0.30 mg).

Discussion

This study presents data on uterine activity in sows, collected with a non-surgical technique which enables passage of the cervix. With this technique, data on uterine pressure variation can be collected in a relatively large number of sows in a relatively undisturbing way, in contrast to studies using surgical techniques (Zerobin & Sporri, 1972; Bower, 1974; Scheerboom et al., 1987). Moreover, in terms of sperm transport, uterine intraluminal pressure might be a more suitable parameter of myometrial activity, because transport of fluids containing sperm cells probably depends on pressure differences in the uterus. Intraluminal pressure variation were only reported by Docke and Worch (1963). Measurement of intra-uterine pressure is, however, limited to the caudal part of a uterine horn. Insertion of the device involves manipulation of the vagina and cervix, which might be expected to affect uterine activity. In the mare, intensive manipulation of the genital tract caused a release of oxytocin (Nikolakopoulos et al., 2000). In pigs (Docke & Worch, 1963; Bower, 1974) and cows (Cooper et al., 1986), manipulation of the genital tract during estrus did not affect uterine motility, except for clitoral stimulation, which elicited a short-lived, reflex-like effect. From preliminary work at our labaratory, it appeared that deliberate manipulation of the device after insertion in the uterine lumen, did not visibly affect myometrial activity, and did not cause a release of oxytocin. Variation due to a varying position of the catheter tip during measurements was not likely, because care was taken to keep the measuring device in the same position throughout a measurement. Moreover, variation in the pattern of myometrial activity was not observed throughout the measuring periods.

In the present study, spontaneous activity of the myometrium, in terms of frequency and amplitude of contractions, increased during the days before estrus, was maximal during estrus, and declined again after estrus. Variation between cycles, especially for frequency of contractions, was large. Before estrus, up to 50 % of the sows did not show any myometrial activity at all, while some sows showed contractile activity with frequencies up to 30 h^{-1}. The absence of uterine contractions in some sows was not caused by a limited sensitivity of the technique: pressure variations as small as 2 to 4 mmHg, caused by respiratory movements of the sows' body could be detected.

Amplitude was relatively low during the days before estrus. During estrus, all sows showed myometrial activity, although variation in frequency and amplitude of contractions was still large.

Changes in average myometrial activity around estrus observed in this study probably reflect endocrine dynamics in the period around estrus and ovulation. Estrogen for example, has been shown to increase gap-junctional coupling (Verhoeff et al., 1986; Garfield et al, 1988), myosin content (Michael & Schofield, 1969) and pace-maker activity (Finn & Porter, 1975) of myometrial cells. Low average frequency of contractions before estrus probably reflects low pacemaker activity, and the relatively low amplitude of contractions before estrus probably reflects decreased cell to cell communication. Sows with a shorter duration of estrus showed an earlier decline in amplitude of contractions, possibly related to an earlier decline in plasma estrogen concentration. Earlier studies on myometrial activity in pigs have also shown an increase in spontaneous activity during estrus (Docke & Worch, 1963; Zerobin, 1968; Bower, 1974; Scheerboom et al., 1987; Claus et al., 1989). All studies report an increase in amplitude and, except for Claus et al. (1989), Docke and Worch (1963), and Zerobin (1968), an increase in frequency of contractions during estrus. Studies are hard to compare quantitatively, because different techniques to record myometrial activity were applied, activity was recorded in different parts of the uterus, and definition of a contraction differs between authors and between techniques. The prominent increase in uterine activity during estrus supports its supposed function in sperm transport and subsequent fertilization. Direction of contraction waves was not recorded in the present study. Zerobin and Sporri (1972), and Bower (1974) reported propagation of contractions along the uterine horns in both tubo-cervical and cervico-tubal direction; the first direction being predominant, especially during estrus. Both authors recorded myometrial activity at 2 sites in the tubal end of the uterine horns. Because the tubal end and the cervical end are supposed to be the regions with the most pacemaker activity (Garfield et al., 1988), it is likely that the majority of the contractions recorded in the tubal end originate in that part and are directed tubo-cervically.

The huge variation in frequency and amplitude around estrus between cycles might in part be explained by 1. variation between sows, and by 2. by diurnal variation. Variation between sows might possibly be due to variation in endocrine dynamics, in receptor populations, or even due to parity. Peri-ovulatory plasma profiles of estradiol-17β, progesterone and LH, and timing of peak levels in LH and estradiol-17β relative to the onset of estrus, differ considerably between sows (Soede et al., 1997). In the present study, onset of standing estrus was used as the reference point in describing change in uterine activity. Therefore, variation between sows in endocrine levels and in timing of endocrine events might be a source of between-sow variation in uterine activity. In studies on myometrial electrical activity (Hoang-Vu, 1987), duration and amplitude of contractions were related to plasma estradiol levels around estrus. Endocrine changes around estrus do not only affect autonomous functioning of the myometrium, but also myometrial receptor dynamics around estrus. Estradiol, progesterone, and oxytocin receptor concentrations (Thilander et al., 1990; Wathes et al., 1996), but also cell to cell contacts in the uterus (Verhoeff et al., 1986) are known to be regulated by estradiol and progesterone. Therefore, variation in spontaneous uterine activity can also be due to variation in sensitivity to circulating hormones. Sensitivity to oxytocin, for example, increases around estrus (Okano & Okuda, 1996; Franczak et al., 2000), but varies between animals in studies with mares (Cadario et al., 1999; Gutjahr et al., 2000).

Diurnal variation might explain part of the variation found between different recordings on the same day of estrus in the present study. In the present study recording continued for only one hour on each day, and therefore variation throughout the day within one sow could not be studied. On the other hand, average uterine activity did not differ between morning and afternoon recordings. In a study with mini-pigs (Taverne, 1982), electrical myometrial activity did show a rythmic fluctuation throughout the day, during a few days before estrus. In a study with Landrace sows (Hoang-Vu 1987), no diurnal variation was recorded in frequency of contractions during estrus. In ewes, plasma oxytocin levels during estrus are low, but show a pulsatile pattern throughout the day (Gilbert et al., 1991), and in pregnant rhesus monkeys, plasma oxytocin levels show diurnal variation that is correlated with diurnal variation in uterine activity (Hirst et al., 1991). Oxytocin is important in mediating

external effectors of uterine activity, like boar odour (Mattioli, 1986) and mating (Claus & Schams, 1990; Soede, 1993), but might also be involved in diurnal modulation of spontaneous activity. However, there is no information on the release of oxytocin throughout the day during estrus in pigs. Boulton et al. (1996) indicated an increase of mRNA encoding oxytocin in the uterus during estrus, although sow numbers were very low in their study. Fluctuating levels of oxytocin, but also diurnal variation in plasma levels of estradiol, might cause diurnal variation in swine, in a direct way but also in an indirect way. Both estradiol and oxytocin have been shown to affect release of prostaglandin by the uterus (Edgerton et al., 2000), thus being able to affect uterine activity in an indirect way. Information on plasma prostaglandin levels during estrus in pigs is scarce. One study (Shille et al., 1979) reports low, but fluctuating levels of prostaglandin during estrus in sows. Therefore, more information is needed to establish the existence of diurnal variation or fluctuation in endocrine levels and myometrial activity during estrus in sows.

Myometrial activity was not affected by infusing a volume of 100 ml of saline, nor by infusion of 100 ml seminal plasma. It was therefore unlikely that the volume of the inseminate affected myometrial activity. However, during natural mating a larger volume of ejaculate is usually deposited into the cervix, and stimulation of myometrial activity cannot be excluded. Bower (1974) reported an almost immediate, positive effect on uterine activity of insemination with semen extender. Claus (1990[a]; 1990[b]) recorded a positive effect of inseminating with 100 ml saline (n = 4) during the second hour after insemination, but proposed that the effect was caused by cervical stimulation with the insemination catheter.

Infusion of estrogens and cloprostenol increased myometrial activity, although there was variation between measurements in the effect of both infusates with respect to the effect on frequency and amplitude of contractions. However, all recordings showed an increase in cumulative amplitude of contractions. Even in sows with minimal myometrial activity, a reaction was elicited after infusion of cloprostenol. Claus (1989) also reported a positive effect of estrogens on frequency of myometrial activity. Both the present study and Claus (1989) applied physiological doses of estrogens. Estrogens in seminal plasma are thought to stimulate myometrial activity, through the release of

prostaglandins by the myometrium (Claus, 1990). Both the fact that the applied amount of estrogens was within a physiological range, and the fact that even sows with minimal activity were stimulated, indicates a possible role for seminal plasma in the control of uterine activity around mating. Infusion of seminal plasma did not affect myometrial activity; this was possibly due to the low estrogen content of the collected seminal plasma (80 pg/ml), as compared to average values reported by Claus (1990). On average, the effect of estrogens and cloprostenol became apparent around 10 min after start of infusion, indicating a fast absorption and fast effectory system. Claus (1990) also reported a rapid rise in estrogens and prostaglandins in the uterine vein after intra-uterine infusion with estrogens. This fast effect on myometrial activity suggests that not only prostaglandin, but also estrogens might act through membrane binding mechanisms, action through nuclear receptors being too slow to explain the fast effects observed in the present study. The effect of both estrogens and cloprostenol was sustained during the entire 1 h of recording after infusion. This is in agreement with Claus (1989), who reported a sustained increase in frequency of contractions, until 3 h after infusion, stressing the possible role of estrogens in seminal plasma.

Clenbuterol suppressed myometrial activity in the present study, reducing both frequency (from 18 to 6 h^{-1}) and amplitude (from 57 to 47 mmHg) of contractions. Clenbuterol acts on β-adrenergic receptors, inhibiting smooth-muscle activity. On average, the effect of clenbuterol became apparent 10 min after infusion, and lasted for the entire 1 h after infusion. Although clenbuterol itself serves no physiological function in fertilization processes, it might provide a tool in studies focusing on the relation between myometrial activity and sperm transport.

In conclusion, this study demonstrates that myometrial activity increases during estrus, with large variation between sows. The differences between sows are consistent throughout the period around estrus. If uterine contractions are important for sperm transport and fertilization, then these differences might explain some of the variation between sows in fertilization rate. Further studies have to be conducted to elucidate the origin of variation in myometrial activity, and its role in fertilization. This study also confirms the effect of estrogens in boar seminal plasma on myometrial activity, as was

earlier shown by Claus (1990). Additionally, the potency of cloprostenol and clenbuterol as agonist and antagonist of myometrial activity in future studies was demonstrated.

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CHAPTER 5

Submitted to Reproduction

Effects of different sexual stimuli on oxytocin release, uterine activity and receptive behaviour in oestrous sows

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SUMMARY

This study was designed to assess the effect of exogenous oxytocin (OT) on uterine activity, and to compare three different sexual stimuli in their effects on OT release, uterine activity and receptive behaviour in oestrous sows. Uterine luminal pressure, as an indicator of uterine activity, was recorded non-surgically, by transcervical insertion of an open-end catheter into the caudal part of the uterine lumen. After recording spontaneous uterine activity for ca 30 min, exogenous OT was administered (Exp 1), or one of the following stimuli was applied to the sow (Exp 2): 1) Tactile stimuli, i.e. manual stimulation of the sow's back and flanks, 2) Tactile stimulation in combination with boar pheromone spray (5α-androstenon), or 3) Tactile stimuli in the presence of a boar. Subsequently, recording of uterine activity continued to assess effects of treatments. Both exogenous OT and endogenously released OT increased uterine activity. The effect was dependent on the uterine activity before treatment, the effect being greater in those sows with lower uterine activity before treatment. In Exp 2, boar presence was the only stimulus that elicited a clear, surge-like release of OT, and also clearly increased uterine activity. Release of OT was not necessary for induction of receptive behaviour: treatments 1) and 2) elicited a standing response in one third of the sows without inducing OT release. Nevertheless, the duration of the standing response in the presence of the boar was related to the magnitude of OT release, indicating a possible role of OT in receptive behaviour.

Key words: Male sexual fuction, Oxytocin, Uterus, Pheromones, Behaviour

Introduction

During courting and mating in pigs, several male sexual stimuli are involved, which are supposed to play a role in the expression of receptive behaviour and in the fertilisation process in sows (Soede et al., 1993). These 'boar stimuli' consist of olfactory, visual, tactile and auditory components. One of the factors mediating the influence of boar stimuli on receptive behaviour and the fertilisation process is probably oxytocin. The presence of a boar before and during mating (Claus and Schams, 1990; Kotwica et al., 1995), and an olfactory component of boar stimuli, 5α-androstenon (Mattioli et al., 1986), have been shown to induce the release of oxytocin (OT) in the sow. Which components of boar stimuli are important and how effective they are in releasing OT is not clear, as there are no studies comparing different stimuli.

Oxytocin is generally accepted to stimulate uterine activity (Finn and Porter, 1975), which might affect the process of sperm transport through the genital tract and fertilisation (Claus, 1990). In pigs, however, there is little information on the in vivo effects of OT on uterine activity during oestrus, and on the role of OT in mediating effects of boar stimuli on uterine activity. Whether OT also plays a role in receptive behaviour in pigs is not known. Studies in species other than the pig indicate that OT has facilitatory and reinforcing functions in sexual behavior (Carter, 1992).

This paper describes two experiments. In the first experiments, the in vivo effects of OT on myometrial activity during oestrus in sows were studied. In the second experiment, three different boar stimuli were compared in their effects on OT release, uterine activity and receptive behaviour and the relationship between the magnitude of OT release and uterine activity and receptive behaviour was studied.

Materials and Methods

Animals and Housing

Experiments were approved by the Ethical Committee for animal experiments of Wageningen University. Eight cyclic, primiparous sows of a commercial breed (Hypor, Euribrid, Boxmeer, The Netherlands), weighing 210 kg on average, were used for both experiment 1 and 2. Animals were housed individually in pens of ca. 3 m × 3 m. During the days of oestrus of every cycle, the sows were housed in crates (2.5 m × 0.8 m), to facilitate measurement of uterine activity and blood sampling. Sows were fed twice daily with a standard gestation diet (2.5 kg per day, 12.9 MJ metabolisable energy kg^{-1}, 130 g crude protein kg^{-1}). Detection of oestrus was performed daily at 0800 and 1600, by applying a back pressure test (BPT) in the presence of a teaser boar. In pigs, the duration of oestrus is about two days on average. During this period, the sow will show a *standing response,* when manual pressure on the back and in the flanks (BPT) is applied in the presence of a boar. Therefore, the period of oestrus is referred to as *standing oestrus.* The standing response indicates receptivity, and is characterised by a frozen stance of the sow, and cocked ears. If a sow shows a standing response upon confrontation with a boar, without additional manual stimuli (BPT), the response is referred to as *spontaneous standing response.*

Measurement of uterine activity

Intra-luminal pressure was recorded on Day 1 and/or Day 2 of standing oestrus (Day 1: first day of standing oestrus). Measurement of uterine intraluminal pressure changes was performed non-surgically by adapting a device used for non-surgical embryo transfer in pigs (Hazeleger and Kemp, 1994). The technique and the procedure for recording uterine pressure are described in detail by Langendijk et al. (2000). The technique consists of passing an open-end, fluid filled catheter through the cervix into the caudal part of the uterine horn. The catheter was removed after each recording session (ca. 2 h), and inserted again at the next recording session. The measuring catheter was connected to a blood pressure transducer (World Precision Instruments, USA), which was positioned at a fixed height, and connected to an amplifier (World

Precision Instruments, USA). The signal from the amplifier was filtered by a low pass filter with a cutoff at 0.23 Hz (kindly provided by J. Korterik, University of Twente, The Netherlands), and recorded on a pen recorder (Pharmacia, Woerden, The Netherlands).

After insertion of the measuring device, spontaneous uterine activity (before treatments) was recorded for 30 to 45 min, before applying one of the treatments as described for experiment 1 and 2. After application of a treatment, recording of uterine activity continued for 1 h in experiment 1 and for 40 min in experiment 2. Uterine activity during this period was characterized by frequency, mean amplitude, and mean duration of contractions. A contraction was defined as a temporary rise (minimum duration 8 sec) in luminal pressure, exceeding 12 mm Hg above baseline pressure. Two consecutive rises in luminal pressure were considered separate contractions, when the second rise occurred after pressure had decreased below 6 mmHg above baseline pressure, for at least ten seconds. These criteria were based on preliminary analyses, and were chosen to discriminate between contractile activity and artifacts in recorded pressure caused by e.g. movement of the sow. Amplitude of a contraction was the highest pressure recorded during the contraction, and expressed in mmHg above baseline pressure. Duration of a contraction was the time between the rise in pressure and the return to baseline pressure. Frequency was expressed in number of contractions per hour. Additionally, cumulative amplitude was calculated as the sum of the amplitude of all contractions in a certain period, and served as a measure for 'total activity'. The effect of a treatment was calculated on an individual sow basis, as the difference between uterine activity before and uterine activity after the treatment.

Experiment 1

For experiment 1, most of the eight sows were used for measurements on more than one occasion: on Day 1 and/or Day 2, during two consecutive oestrous periods. Per sow, not more than one measurement was performed per day. Measurements did not start before 1000, to avoid effects of boar presence during the detection of estrus at 0800. On average, two observations were obtained on each sow. After recording spontaneous uterine activity for 30 to 45 min, sows were either injected with 5 IU OT

i.m. (N = 22), or 2 IU OT i.v. (N = 6) (Intervet, Boxmeer, The Netherlands), and recording continued for one hour to assess effects. For i.m. injection, sows were allowed to remain in the position they had adopted before injection, i.e. lying or standing. For i.v. injection, sows were forced to stand up, immobilized by means of a nosesling and injected into an ear vein using a 21 G winged infusion set. Previous work (unpublished results) showed that immobilization by means of a nosesling during 5 min did not affect uterine activity during 60 min post treatment.

Experiment 2

Before the start of experiment 2, the eight sows were surgically fitted with an indwelling jugular catheter, as described by Soede et al. (1997). After surgery, catheters were flushed twice a week with sterile, heparinised saline to prevent obstruction.

Treatments. For experiment 2, the eight sows were also used for measurements on more than one occasion: on Day 1 and/or Day 2, during several consecutive oestrous periods. Per sow, not more than one measurement was performed per day. Measurements did not start before 1000, to avoid effects of boar presence during the detection of estrus at 0800. On average, six observations were obtained on each sow, and these (N = 47) were distributed as equally as possible among Treatment × Day combinations. After recording spontaneous uterine activity for 30 to 45 min, one of three treatments was applied: 1) **BPT**, Back Pressure Test : the sow was gently urged to stand up, and by rubbing and pushing the sow in the flanks and on the back, the tactile stimuli of a boar around mating were mimicked during 1 min, trying to induce a standing response. 2) **BPT+Spray.** After having urged the sow into a standing position, boar pheromone (5α-androstenon, Boar Mate, Antec International, UK) was sprayed during 2 sec from a distance of 30 cm from the nose of the sow (T0). The sow was observed for a spontaneous standing response (without additional manual stimuli). After 1 min, a BPT was performed as for 1), regardless of whether the sow showed a spontaneous standing response. 3. **BPT+Boar.** A teaser boar was introduced in front of the sow pen (T0), and allowed to have nose-to-nose contact with the sow. The sow was observed for a spontaneous standing response, and after 1 min a BPT was

performed as for 1) and 2) The boar was allowed in front of the sows during 5 min and then removed again from the barn. For all three treatments, the time during which a standing response was exhibited, was recorded.

Blood sampling procedure. Because of obstructed catheters, blood samples were obtained during 35 of the 47 measurements, being equally distributed over Treatment × Day combinations. Blood samples (5 ml) for OT analysis were taken at 10 and 5 min before a treatment, and at short intervals following the start of a treatment (T0): at 1 min intervals from T0 to T10 min, at 2 min intervals from T10 to T30 min, and at 5 min intervals from T30 to T60 min. Blood was collected in heparinized tubes, kept on ice, centrifuged (3000 rpm, 10 min) and stored at –20 °C. To assess the effect of introduction of the open-end measuring catheter through the cervix into the uterus on OT release, on four different occasions, blood samples were taken at 5 min intervals, from 20 min before until 45 min after introduction of the catheter.

Oxytocin assay. Oxytocin was determined by radio immunoassay as originally described for cattle (Schams et al., 1979) and with a method of improved sensitivity (Schams, 1983), after extraction with SEP-PAK C18 cartridges (Waters, Milford, MA 01757, USA). The antiserum did not cross react with related peptides such as lysine- or arginine- vasopressin or anterior pituitary hormones. The assay was validated for pig plasma. The extraction recovery at 0.8, 1.6, 3.2 and 6.4 ng/l was 78 ± 9 % (mean ± SD for n = 7). The within-assay CV varied from 5.2 to 8 % and the between-assay CV from 10.4 to 15.2 % in samples with high (20.2 ± 1.8 ng/l) and low (1.5 ± 0,2 ng/l) OT concentrations.

Statistical analyses

Data were analyzed separately for experiment 1 and 2, using SAS (1990). In both experiments, effects of the treatments were calculated per measurement as the difference between uterine activity before and after a treatment. Thus, the change in frequency, mean amplitude, and mean duration of contractions, and the change in cumulative amplitude were calculated and fitted to the following model: $Y_{ij} = \mu + T_i + D_j + f_{spont} + e_{ij}$; with Y_{ij} the change in one of the uterine activity parameters, T_i the

treatment, D_j the day of oestrus, and f_{spont} the frequency of contractions before a treatment. The latter was included as a covariate in the model. Separate measurements obtained from one sow were regarded as independent, because the number of observations was too low to establish a sow-effect with treatments nested within sows. In experiment 1, effects of 2 IU OT i.v. on frequency and amplitude of uterine contractions were not different from 5 IU OT i.m. Data were therefore pooled. In experiment 2, the effect of treatment and day of oestrus on the exhibition of a standing response (yes/no), was tested with a χ^2 test. Data on OT release were fitted to the following model: $Y_{ij} = \mu + T_i + S_j + T_i{*}S_j + e_{ij}$, with Y_{ij} the level of OT, T_i the treatment, and S_j the sample number (i.e. the time relative to the onset of the treatment). LSD-tests were performed to compare oxytocin levels between treatments at the same sampling time, only after ANOVA pointed out that there was an interaction between sampling time and treatment. For pre-planned comparison of LS-means, Bonferroni correction was applied to control overall type I error.

Results

Experiment 1

Frequency of spontaneous uterine contractions was 19 ± 1.4 h^{-1} (0 to 33 h^{-1}), and mean amplitude of spontaneous contractions was 60 ± 5.1 mmHg (24 to 105 mmHg). Injection with OT increased only frequency (n = 2), only amplitude (n = 7), or both frequency and amplitude of contractions (n = 19. In two cases no spontaneous uterine contractions were recorded. In these cases, myometrial activity was triggered towards 12 and 22 contractions per h by OT. Overall, frequency of contractions increased with 5 ± 1.5 h^{-1} (- 6 to + 22 h^{-1}; $P < 0.01$), and mean amplitude of contractions increased with 32 ± 5.8 mmHg (-13 to +96 mmHg; $P < 0.01$). The change in frequency of contractions was related to the frequency of spontaneous uterine contractions before treatment (Fig. 1). For sows with below average spontaneous frequency of contractions the increase in frequency was 9 ± 1.9 h^{-1} ($P < 0.05$); for sows with above average spontaneous frequency of contractions OT had no effect on frequency of contractions. The change in amplitude of contractions was not related to spontaneous uterine (frequency nor amplitude) before treatment with OT. Cumulative amplitude,

i.e. the sum of the amplitude of all contractions during 1 h, was increased by OT in all cases with + 1159 mmHg.h^{-1} on average (+153 to +2100 mmHg.h^{-1}; $P < 0.01$). Mean duration of contractions was not affected by OT treatment. Day of oestrus was not related to frequency or amplitude of contractions, nor to the change in frequency or amplitude after injection of OT.

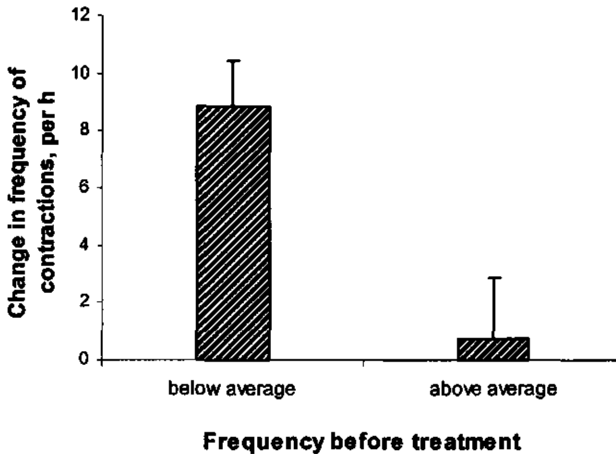

Figure 1. The mean ± SE increase in frequency of uterine contractions after injection with oxytocin, for sows with a below average or above average frequency of contractions before the treatment. Average frequency before treatment was 19 h^{-1}. Sows were injected with either 5 IU oxytocin im (n=22) or 2 IU oxytocin iv (n=6). There was no difference between the two ways of administering oxytocin, and therefore the data were pooled. * Significant difference between the two groups of sows (P < .05).

Experiment 2

On the first day of oestrus (Day 1), only the presence of a boar ($P < 0.01$) induced a standing response (Table 1). In four of the eight cases, exposure to the boar even induced a spontaneous standing response, i.e. before applying tactile stimuli. On Day 2, the sows were more receptive to the applied stimuli: both tactile stimuli (BPT) and tactile stimuli in combination with olfactory stimuli (BPT + Spray) induced a standing response in about 50 % of the cases. Exposure to the boar induced a spontaneous standing reponse in seven of the eight cases. Overall, boar presence was a more

potent stimulus in evoking a standing response, compared to the other two stimuli ($P < 0.01$).

Table 1. Number of sows showing estrous behavior in reaction to three different levels of boar stimuli, on the first and second day of estrus.

Day of estrus		BPT[1]	BPT + Spray[2]	BPT + Boar[3]
First	N	8	8	8
	Sows with SR[4]	0	0	8
	Sows with spontaneous SR[5]	-	0	4
Second	N	7	8	8
	Sows with SR	4	5	8
	Sows with spontaneous SR	-	1	7

[1]BPT: Back pressure Test, manual pressure on the back of a sow to evoke a standing response (receptive behaviour. [2]BPT + Spray: boar pheromone spray (5α-androstenon), in combination with a BPT, 1 min after spray. [3]BPT + Boar: Presence of a boar during 5 min, in combination with a BPT, 1 min after entrance of the boar. [4]SR: Standing response. [5]Spontaneous SR: standing response shown before applying the BPT.

Basal levels of OT, i.e. the level recorded before applying a treatment, varied between 1 and 5 pg/ml (Fig. 2). In all four observations where blood was sampled around introduction of the measuring catheter, introduction did not induce a release of OT. In one of the four observations, OT level increased slightly to 23 pg/ml, but this was 35 min after introduction of the catheter. Boar presence in combination with tactile stimuli caused a clear, immediate, surge-like release of OT in all sows, with peak levels ranging from 11 to 189 pg/ml (average 80 pg/ml). Oxytocin concentrations rose above basal level within 1 min for these sows, and maximum OT levels occurred between 1 and 3 min following introduction of the boar. Oxytocin levels returned to basal levels 20 to 30 min later. Mean OT peak in the presence of the boar tended to be higher on Day 2 than on Day 1 (102 pg/ml vs 58 pg/ml; $P = 0.13$). However, a comparison within those sows that had observations on both Day 1 and Day 2, showed that the pattern of OT release was not related to day of oestrus. Tactile stimuli (BPT) and tactile stimuli in combination with boar pheromone spray (BPT + Spray), did not have any effect on OT levels. Only in one case with the BPT treatment, a small OT peak (12 pg/ml) was observed.

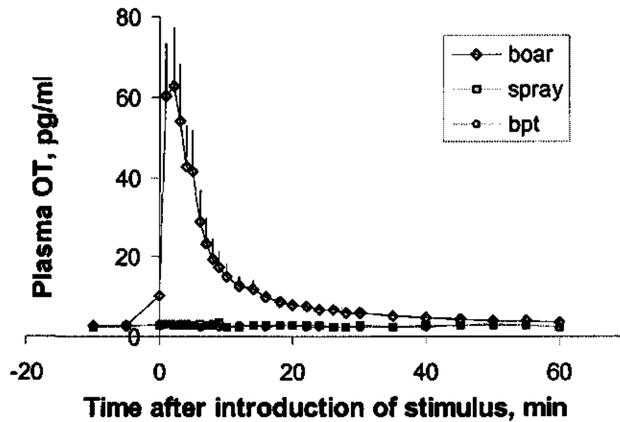

Figure 2. Mean (± SE) plasma oxytocin levels per stimulus before and after application of three different boar stimuli: 1. BPT (n=11): back pressure test, i.e. manual stimulation of the back and the flanks of a sow, 2. SPRAY (n=12): a back pressure test in combination with boar pheromone spray, 3. BOAR (n=12): a back pressure test in the presence of a boar. T=0 is the time of commencement of a treatment.

Tactile stimuli (BPT and BPT + Spray Treatments) induced a standing response that lasted as long as the tactile stimuli were applied, except for one case where pheromone spray already induced a spontaneous standing response before tactile stimuli were applied. The standing response for these treatments disappeared when tactile stimulation ceased, and therefore, the duration of the standing response was maximal at 1 min. In the presence of a boar the standing response commenced as early as the entrance of the boar (T0), if a spontaneous standing response was induced, and could last until around the time the boar was removed. Removal of the boar terminated the standing response in those sows still showing a standing response at that time. The duration of the standing response in the presence of the boar, therefore mounted up to 6 min. The duration of the standing response in the presence of the boar was related to the peak in OT release (Fig. 3). The duration of the standing response increased with the magnitude of OT release reaching a plateau at about 75 pg/ml OT. Above this level of OT release, the duration of the standing response was at least 4 min.

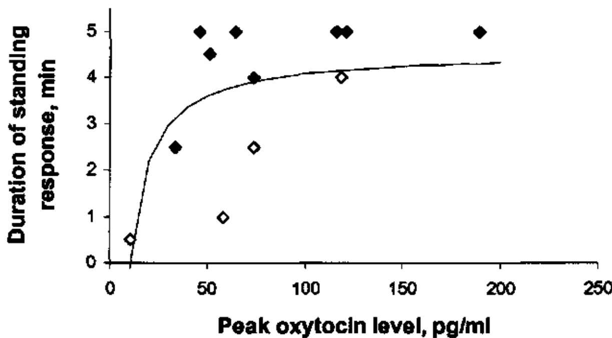

Figure 3. Duration of the standing response in the presence of a boar in relation to the peak in plasma oxytocin release, for individual sows. A standing response was shown either spontaneously after confrontation of the boar to the sow (closed symbols), or after an additional back pressure test (manual stimulation of the back and the flanks of the sow). The boar was removed after 5 min, which in most cases ended the standing response of the sows. Regression line: y= 4.6 - 47.4/x (R^2 = 0.49).

Frequency of spontaneous uterine contractions was 25 ± 1.7 h^{-1} (15 to 39 h^{-1}), and mean amplitude of spontaneous contractions was 59 ± 4 mmHg (29 to 104 mmHg). Overall, tactile stimuli (BPT), boar pheromone (BPT + Spray) and boar presence caused a slight increase in frequency of contractions (+ 2 h^{-1}; $P < 0.10$, +1 h^{-1}; NS, and + 3 h^{-1}; $P < 0.05$, respectively), and a slight increase in mean amplitude of contractions (+ 7 mmHg; $P < 0.10$, +6 mmHg; NS, and + 10 mmHg; $P < 0.01$, respectively). The change in frequency was dependent on the frequency of spontaneous uterine contractions (Fig. 4). For those cases with frequency of spontaneous contractions below 25 h^{-1} (ca 50 %), frequency of contractions was clearly increased by boar presence (+7 h^{-1}; $P < 0.05$), and to a small extent by tactile (+3 h^{-1}; $P < 0.05$) and pheromonal (+3 h^{-1}; $P < 0.05$) stimulation. The effect of BPT + Boar was greater than the effect of the other two treatments ($P < 0.05$) for the cases with frequency of spontaneous contractions below 26 h^{-1}. The change in amplitude of contractions was not affected by uterine activity before treatments. Mean duration of contractions was not affected by any of the treatments. Cumulative amplitude, i.e. the sum of the amplitude of all contractions during one hour, was increased in all cases for

the BPT + Boar treatment: +461 mmHg.h^{-1} (+24 to +1327 mmHg.h^{-1}; $P < 0.01$). For the BPT treatment, cumulative amplitude was increased with +313 mmHg.h^{-1} (-701 to +984 mmHg.h^{-1}; $P < 0.05$). For the BPT + Spray treatment, cumulative amplitude was increased with +211 mmHg.h^{-1} (-325 to +747 mmHg.h^{-1}; $P < 0.10$).

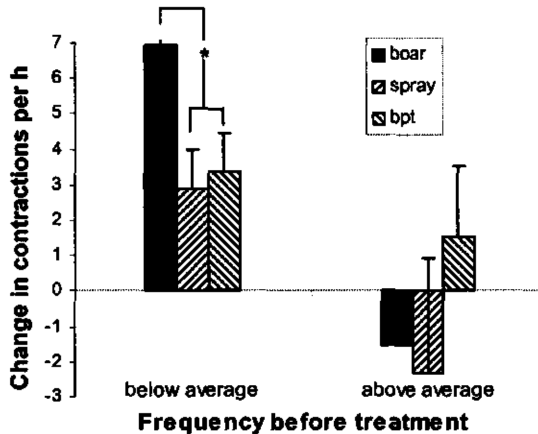

Figure 4. The mean ± SE increase in frequency of uterine contractions after one of three different stimuli, for sows with a below average or above average frequency of contractions before the treatment. Average frequency before treatment was 25 h^{-1}. The three different stimuli were: 1. BPT (n=15): back pressure test, i.e. manual stimulation of the back and the flanks of a sow, 2. SPRAY (n=12): a back pressure test in combination with boar pheromone spray, 3. BOAR (n=15): a back pressure test in the presence of a boar. * Significant difference between diferent stimuli ($P < .05$). In the sows with above average frequency of contractions before treatment, the treatments had no effect. In the sows with below average frequency of contractions before treatment, all three stimuli increased frequency significantly ($P < .05$), but the effect of BOAR was greater than the other two stimuli ($P < .05$).

Uterine activity (frequency and amplitude of contractions) before treatments, was not related to the basal levels of OT measured before treatments. Uterine activity after treatment was not related to OT levels, in any of the three treatments. In the presence of the boar, the magnitude of OT release was not related to the change in frequency of contractions. Day of oestrus was not related to frequency or amplitude of contractions, nor to the change in frequency or amplitude after treatments.

Discussion

This is the first study in sows comparing different boar sexual stimuli in their ability to induce the release of oxytocin (OT), and in their effects on myometrial activity. The presence of a boar appeared to be more potent in inducing the release of OT: tactile stimuli, or a combination of tactile and olfactory stimuli did not induce any release of OT. The pattern of OT release in the presence of the boar was comparable to the release of OT in mated sows (Claus and Schams, 1990; Kotwica et al., 1995), although in the latter case OT levels remained elevated during a longer period of time (60 min vs 30 min). In contrast to the present study, Mattioli et al. (1986) reported a clear release of OT (peak values 24 to 101 pg/ml) induced by boar pheromone spray, which is in the same range as the values recorded in the presence of the boar in our study. Both Mattioli et al. (1986) and the present study used a 5α-androstenon containing odor, sprayed during 2 sec. The concentration of 5α-androstenon in the aerosol used in the present study, however, was about two-thirds of the concentration used by Mattioli et al (1986). The way detection of oestrus was performed might also be an explanation for the contradictory results. Mattioli et al. (1986) do not mention the daily use of a boar for detection of oestrus, as was used in our study. Regular contact with a boar can cause habituation to boar stimuli, reducing responsiveness (exhibition of receptive behaviour) to lower levels of boar stimuli (Hemsworth and Hansen, 1990; Langendijk et al., 2000). The use of a boar for detection of oestrus might have caused a decreased sensitivity to the other stimuli in the present study. Whether this could have affected the ability of stimuli to induce OT release is not clear. The extreme difference in OT release between the presence of the boar and the use of pheromone spray suggests that other factors also play a role, as desensitization would more likely have caused a gradual difference in OT release.

Injection with OT, either 5 IU i.m. or 2 IU i.v., caused an increase in uterine activity, either by increasing frequency (+ 5 h^{-1}) or amplitude (+ 32 mmHg) of contractions, or both. The effect was dependent on the uterine activity before treatment; for sows with a lower frequency of contractions the increase in uterine activity was greater (+ 9 h^{-1}). Thus, treatment with OT selectively stimulated uterine activity, mainly in those sows with lower uterine activity before treatment. Effects of tactile and olfactory stimuli on

uterine activity were negligible, whereas the presence of the boar had a clear effect on uterine activity. Thus, the effect of the three stimuli on uterine activity paralleled the effect of the three sexual stimuli on the release of OT. The effect of the presence of the boar was comparable to the effect of injection with OT: for sows with lower spontaneous uterine activity, the effect of OT or boar presence was greater. As for injection with OT, the increase in uterine activity after introduction of the boar was more pronounced for sows with lower uterine activity (+ 7 h^{-1} for sows with lower uterine activity vs + 3 h^{-1} for the average sow). If uterine activity promotes sperm transport and fertilisation, these results suggest a mechanism which promotes uterine activity in those sows where sperm transport might otherwise be a limiting factor for fertilisation. In situations that are suboptimal for fertilisation, OT has been shown to increase fertility. In swine, administration of OT compensates for seasonal effects on fertility (Pena et al., 1998), and improves results of inexperienced inseminators (Flowers, 1994). In infertile women, OT has been shown to increase chance of conception in those cases where uterine activity responded positively to OT administration (Wildt et al., 1998).

As for the induction of OT release, the presence of a boar was also a more potent stimulus in evoking oestrous behaviour (standing response) than tactile stimuli and olfactory stimuli. Presence of a boar elicited a standing response in all sows. Tactile and olfactory stimuli elicited a standing response in only one third of the sows. Olfactory stimuli, especially 5α-androstenon, are an important component of the complex of boar stimuli, and have been shown to elicit a standing response in at least a percentage of sows that did not respond to tactile stimuli (Melrose et al., 1971). However, components of boar stimuli are not as potent as a whole boar in evoking receptive behaviour, as has been shown in studies confronting sows both to components of stimuli and to a whole boar (Signoret, 1970; Perry et al., 1980; Pearce and Hughes, 1987). Olfactory stimuli did not add to the potency of tactile stimuli to induce receptive behaviour in the present study. As has been pointed out before, responsiveness to components of boar stimuli might be reduced in a situation where sows have regular contact with a 'whole boar'.

Besides its peripheral effect on the genital tract, the function of OT as a neuropeptide in regulating sociosexual behaviour, has been pointed out for several species (Carter, 1992). Oxytocin probably has a function in several aspects of sociosexual behaviour. These include the reduction in aggression between individuals preceding mating (Witt, 1995), facilitation of receptive behaviour, sexual arousal, orgasm, and sexual satiety (Carter, 1992). Similarly, OT might have a role in swine sexual behaviour. The present study shows that the release of OT is not a necessary factor for showing a standing response: the sows that showed a standing response in reaction to tactile and/or olfactory stimuli did not show OT release. On the other hand, the OT release in the presence of the boar coincided with a higher percentage of the sows showing a standing response (100 % vs 30 %), with a longer average duration of that standing response (4 min on average). Moreover, a higher release of OT was related to a longer duration of the standing response in the presence of the boar. Therefore, OT might have a facilitatory role in the expression of receptive behaviour. In gilts, treatment with an antagonist of opioid receptors increased the OT release and the time during which receptive behaviour was shown around mating, whereas an agonist of opioid receptors had the opposite effect on OT release and receptive behaviour (Kotwica et al., 1995). Therefore, the magnitude of OT release seems to be related to the expression of receptive behaviour in swine, although other neuroendocrine factors, probably also play a role. More evidence is needed to establish the regulation of receptive behaviour in pigs.

In summary, the effect of both exogenous and endogenously released OT on uterine activity in oestrous sows depends on the uterine activity before treatment, the effect being greater in those sows with lower uterine activity before treatment. The presence of a boar induces both oestrous behaviour and a clear OT release in oestrous sows, and promotes uterine activity. In contrast, olfactory and tactile stimuli did not induce a release of OT in the present study, and hardly affected uterine activity. Although these latter stimuli were also much less potent in inducing receptive behaviour, OT was not necessary for inducing receptive behaviour. This study provides some indication for a function of OT in sexual behaviour, in that a higher degree of OT release was related to an increased duration of receptive behaviour. More research is needed to support this function of OT.

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CHAPTER 6

Submitted to Reproduction

Functions of myometrial activity in sperm transport through the genital tract and fertilisation in sows

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Summary

This paper describes the effects of stimulation and suppression of uterine contractility around insemination on sperm distribution and fertilisation in multiparous sows. For assessment of fertilisation, sows were inseminated around 28 h before (synchronised) ovulation, and slaughtered 5 days after ovulation (N = 53). For assessment of sperm distribution, sows were inseminated around 20 h before expected ovulation, and slaughtered 12 h later (N = 26). Ten minutes before insemination, sows were infused intra-uterine, with one of three solutions: 1) Saline (control), 2) 0.60 mg Clenbuterol-HCl to suppress contractility, or 3) 1 mg Cloprostenol to stimulate contractility. Both clenbuterol and cloprostenol reduced median fertilisation rate ($P < 0.05$) and median number of accessory sperm cells ($P < 0.05$). Sperm cell distribution was also affected by treatments. Clenbuterol increased, and cloprostenol decreased the number of sperm cells ($P < 0.05$) in the proximal 20 cm of the uterine horn and in the utero-tubal junction (UTJ). The number of sperm cells in the isthmus followed the same trend ($P < 0.10$). However, relative to the number of sperm cells in the uterus, clenbuterol reduced the number of sperm cells in the UTJ and oviduct, in contrast to cloprostenol. Cloprostenol increased the reflux of semen during insemination. It is hypothesised that suppression of uterine contractility increases trans-uterine transport time, reducing the ability of sperm cells to enter the UTJ and the oviduct. Stimulation of uterine contractility above a certain level probably increases reflux and impedes trans-uterine transport of sufficient numbers of sperm cells.

Key words: Uterus, sperm, fertilisation

Introduction

In swine, sperm cells have to be transported from the cervical end to the tubal end of the uterine horns after mating or artificial insemination. The length of the uterine horns averages around 0.8 m during oestrus (Palmer et al., 1965), and the transport of

semen through the horns is believed to be a passive process. This passive transport is probably driven by the flow of intra-uterine fluid containing sperm cells, due to gravitational force, movement of the sow, and uterine contractions (Scott, 2000). The uterine lumen is a relatively hostile environment for the sperm cells, and uterine contractions are believed to be important for transporting sperm cells to the utero-tubal junction (UTJ) as soon as possible after insemination. The UTJ and the first part of the isthmus serve as a barrier for sperm cells and act as sperm reservoirs until around the time of ovulation, when the ascent of sperm cells from the reservoir into the oviduct is increased (Hunter, 1981). Stimulation of uterine contractility is supposed to accelerate the transport of sperm cells to the sperm reservoirs, thus increasing the number of sperm cells surviving until the time of fertilisation. However, there is almost no evidence for the effect of increased uterine contractility on distribution of sperm cells through the genital tract and on fertilisation (Stratman et al., 1959; Baker et al., 1968). Furthermore, there is no literature available concerning the question whether uterine contractility is necessary at all for transport of sperm cells and fertilisation.

Recent research proved the effects of clenbuterol as a suppressor, and cloprostenol as a stimulator, of uterine contractility during oestrus (Langendijk et al., unpublished). The objective of this study was to investigate the effects of these hormones around the time of insemination on the distribution of sperm cells throughout the genital tract, and on fertilisation.

Materials & Methods

Animals and Housing

Experiments were approved by the Ethical Committee for animal experiments of the Wageningen University. On the day of weaning (Day 0), multiparous sows (third to sixth parity) of a commercial breed (Landrace x Yorkshire crossbred) were transported to the experimental farm, in batches of 18 sows. The average lactational period was *21* d (SD *3.4* d), average litter size was *9.8* (SD 1.3), and the sows weighed *235* kg (SD *15.4* kg) on average at weaning. The sows were housed in individual crates and fed twice daily, at 0830 and 1630, and received 2.5 kg per day of a standard gestation diet

(12.9 MJ ME kg^{-1}, 130 g CP kg^{-1}). On Day 3, 83 to 87 h after weaning, the sows were treated with 50 µg of GnRH i.m. (Depherelin®, Veyx-Pharma, Germany), to synchronize ovulation. Preliminary work (Langendijk et al., 2000) showed that in sows treated around this time, ovulation occurs 38 h (35 to 41 h) after GnRH. Per batch, two thirds of the sows were used for recovery of embryos and unfertilised oocytes at 5 days after ovulation, and one third of the sows were used for recovery of sperm cells after insemination.

Treatments

Sows were assigned to one of three treatments around insemination, with parities and body weights distributed equally among the treatments. For recovery of embryos and oocytes, sows were inseminated 28 h before expected ovulation, with 0.5×10^9 sperm cells in 40 ml. Timing and dose were chosen to achieve intermediate fertilisation rates for the control treatment. For recovery of sperm cells, sows were inseminated 20 h before expected ovulation, with 3×10^9 sperm cells in 40 ml. Time and dose in this case were chosen to achieve quantifiable numbers of sperm cells in the genital tract. Semen was obtained from 20 different boars, distributing the boars equally among the three treatments. Insemination was performed with a 'hands free' method, using regular Spirettes (Minitub, Germany), with a *40 cm* tube connecting the spirette to the semen container. The semen container was always fixed at the same height (*ca 25 cm*) relative to the sow's back, to make sure gravitational pressure was constant for all sows. Ten minutes before the start of insemination, the sows were infused with one of the following solutions: (1) 1 mg of Cloprostenol (Estrumate, Schering-Plough, The Netherlands) in 40 ml saline, to stimulate uterine contractility; (2) 0.60 mg Clenbuterol-HCl (Planipart, Boehringer-Ingelheim, The Netherlands) in 40 ml saline, to suppress uterine contractility; or (3) 40 ml of saline (control). Effects of cloprostenol and clenbuterol on uterine activity were established in a previous study (Langendijk et al., unpublished). The solutions were infused in the same way as described for the semen. Time needed for infusing the solutions and for infusing the semen dosages were both recorded. Reflux of semen during insemination was recorded on a subjective scale as no, little (up to ca 5 ml), or much reflux (more than 5 ml). After infusion of the semen 10 ml BTS was infused.

Oestrus and ovulation

Detection of oestrus was performed using a mature teaser boar. On Day 0, Day 1, and Day 2, the sows were exposed to the boar once daily. Starting on Day 3, detection of oestrus was performed at 0600, 1400, and 2200, until all signs of behavioural oestrus had disapperared. The timing of detection of oestrus was chosen to avoid contact with the boar around the time of insemination, which was 1030 (for later embryo recovery) or 1830 (for later sperm cell recovery). Oestrus was regarded to be the period during which the sows exhibited a standing response in reaction to the presence of a boar in combination with manual stimulation of the back and the flanks. Onset of oestrus was defined as 4 h before the first time a standing response was observed. End of oestrus was defined as 4 h after the last time a standing response was observed.

Transrectal ultrasonography was performed using a 7.5 Mhz annular array sector probe (Pie Medical, Maastricht, The Netherlands), according to the method of Soede et al. (1992). Ultrasonography was performed on Day 0, to detect lactational oestrus (corpora lutea on the ovary) and premature oestrus (follicles > 5 mm on Day 0), and on Day 3 to assess follicular development. Only sows not showing lactational oestrus, premature oestrus or lack of follicular development on Day 3, were used for the experiments. On Day 5, ultrasonography was performed at 0630, 1030, 1430, 1830, and 2230, to detect timing of ovulation for the sows in which fertilisation rate was assessed. Time of ovulation was defined as the time between the last time preovulatory follicles were detected and the first time no follicles could be detected on the ovary.

Embryonic development

For recovery of embryos and unfertilised oocytes, the sows were slaughtered at 113 h (109 – 117 h) after ovulation. Collection of the embryos and the oocytes, morphological assessment and further processing and staining for the counting of embryonic nuclei and accessory spermatozoa were performed as described by Steverink et al. (1998). An oocyte was classified as unfertilised if the nuclei count was

zero or one. Embryos with a low number of nuclei, of degenerated appearance, were classified as degenerated embryos. The remaining embryos were considered normal. Recovery rate per sow was calculated as the percentage of embryos and oocytes recovered, based on the number of corpora lutea. Fertilisation rate was calculated as the percentage of normal and degenerated embryos relative to the total number of recovered embryos and oocytes per sow. Number of cell cycles, as a measure of embryonic development, was calculated as the $^{2}\log$ of the number of counted nuclei. The number of accessory sperm cells in the zona pellucida of normal embryos, as a measure of the number of spermatozoa present around the time of fertilisation, was expressed as the average per sow.

Sperm recovery

For the recovery of sperm cells from the genital tract the sperm used for insemination was labeled with Hoechst 33342 (an epifluorescent, non-lethal dye), by adding 200 µl of a 2 mM stock solution to each semen dose, one hour before insemination. Around 8 h before expected ovulation (12 h after insemination), the sows were slaughtered for recovery of sperm cells. Immediately after stunning and exsanguination, the genital tract was removed by abdominal incision, avoiding manipulation of the tract as much as possible, and spread out on a flat surface. The following sections were separated from the rest of the genital tract: the proximal (tubal end) 20 cm of the uterine horns, 1 cm proximal to 1 cm distal from the utero-tubal junction (UTJ), the distal half of the oviduct (isthmus), and the proximal half of the oviduct (ampulla). The proximal 20 cm of the uterine horns were flushed twice with 20 ml Dulbecco's PBS (D-PBS). The isthmus and ampulla were flushed with 5 ml D-PBS, in uterine direction. The UTJ was filled with 1 ml D-PBS, massaged, emptied, and flushed with 5 ml D-PBS in uterine direction. The UTJ was then cut open along the longitudinal axis, turned inside out and rinsed in a 5 ml volume of D-PBS. All fluids were pooled per segment, and centrifuged to concentrate the sperm. Sperm cell concentration in the samples was determined in duplicate, using a Bürker counting chamber. The flushed UTJ, isthmus and ampulla sections were stored at -20 °C until further processing.

After thawing, the UTJ, isthmus and ampulla were homogenised using small surgical scissors. During homogenisation, the tissue was regularly flushed over a 100 µm polyamide sieve with D-PBS, up to a volume mounting of ca 30 ml. After thorough homogenisation, the tissue was suspended in 5 ml D-PBS, vortexed, and flushed again over the sieve with 5 ml D-PBS. The latter procedure was then repeated. The resulting filtrate (ca 50ml) was concentrated to ca 5 ml by centrifugation. Counting of sperm cells was performed using a Bürker counting chamber, under a fluorescence microscope (Zeiss, 200x, UV-filter). Number of recovered sperm cells obtained by the different procedures were added up per genital tract section. In a preliminary trial, with live sperm cells being infused in freshly obtained oviducts in quantifiable amounts (10 000 to 50 000), incubated at 37 °C for ca 1 h and then frozen, this procedure resulted in recovery of around 80 % of the infused sperm cells.

Statistical analyses

Data were analysed using SAS (SAS Inst. Inc., Cary, NC). Effects of treatments on onset of oestrus, duration of oestrus, time of ovulation, number of embryonic nuclei, and the difference between hormone and semen infusion time were analysed by ANOVA using the following model: $Y_{ij} = \mu + T_i + e_j$, with T being the different treatments. Effects of treatments on fertilisation rate, number of accessory sperm cells, number of sperm cells in the different part of the genital tract, time needed for infusion of hormone and semen were analysed using Wilcoxon rank scores in the NPAR1WAY procedure. Correlation between e.g. uterine horn length and number of sperm cells were analysed with ANOVA after log transformation of the number of sperm cells. χ^2 analysis was used to assess relationships among class variables.

Results

Sperm cell distribution

Oestrus. Nineteen of the 26 sows exhibited behavioural oestrus before being slaughtered for recovery of sperm cells. All sows exhibiting oestrus were in oestrus

before or at the time of insemination: the interval from the onset of oestrus to insemination was 12 ± 2 h (0 to 31 h) on average. The onset of oestrus (91 ± 2 h after weaning) was not affected by the treatments.

Recovery of sperm cells. The number of sperm cells counted in the flushings and homogenised samples of the isthmi and ampullae was low; for ca 90 % of the samples, the number of sperm cells counted per Bürker counting chamber was lower than 10. In the UTJ and uterine horn, counted numbers ranged between 0 and 200 per chamber. No sperm cells were recovered in 4 out of 26 sows (ampulla), 4 out of 26 (isthmus), 0 out of 26 (UTJ), and 1 out of 26 (uterine horn). The number of recovered sperm cells was skewed, and varied enormously between sows (Table 1). Most sperm cells were recovered from the UTJ and the uterine horn (both about 50 %), and a small percentage of the recovered sperm cells originated from the oviduct (less than 1 %).

Table 1. Number of sperm cells (x 1000) recovered from the different sections of the genital tract of sows 12 h after insemination with 3 billion sperm cells, by flushing and by homogenising the flushed tissue.

Section		N	Recovery per sow (x 1000)		Sows with no recovery (n)	No. of cells represented by 1 count in cytometer
			Median	Range		
Ampulla	Flushing	26	0.089	0 - 6.3	9	186
	Homogenate	26	1.910	0 - 90.0	6	2408
Isthmus	Flushing	26	0.385	0 - 17.0	5	150
	Homogenate	26	1.928	0 - 13.9	7	870
UTJ	Flushing	26	332.753	1.4 - 1885	0	4000
	Homogenate	26	46.001	2.7 - 552	0	864
Uterine horn	Flushing	26	331.286	0 - 19210	1	21172

Effect of treatments. The number of sperm cells recovered from the different sections of the genital tract was 3549 for the ampulla, 2602 for the isthmus, 557 651 for the UTJ, and 290 521 for the proximal uterine horn (Table 1 and 2). The number of sperm cells in the isthmus was higher for the clenbuterol treatment, compared to the cloprostenol treatment ($P < 0.10$). In the UTJ and the uterine horn, the number of sperm cells was higher for the clenbuterol and the control treatments ($P < 0.05$), compared to the cloprostenol treatment. When numbers of sperm cells in the oviductal segments and the UTJ were expressed relative to the number found in the proximal part of the uterine horns, the treatments had an opposite effect. Per 100 sperm cells in the proximal uterine horns, 43 sperm cells were recovered from the UTJ for the clenbuterol treatment, as opposed to 134 and 201 sperm cells ($P < 0.05$) for the control and the cloprostenol treatment. Similarly, per 100 sperm cells in the horns 0.4 sperm cells were recovered from the oviduct for the clenbuterol treatment, as opposed to 8 sperm cells ($P < 0.10$) for the cloprostenol treatment. The timing of onset of oestrus did not affect the number of sperm cells in any of the segments.

The length of the uterine horns was dependent on the parity of the sows ($P < 0.05$): for third, fourth, fifth and sixth parity sows average horn length was 59 ± 6 cm, 63 ± 8 cm, 76 ± 5 cm, and 86 ± 4 cm. The length of the uterine horn was not related to the number of recovered sperm cells. There was no consistent difference between the left and right side of the genital tract in number of recovered sperm cells, but the number of sperm cells varied considerably between both sides. The average difference between the two sides was 39 %, 47 %, 48 %, and 60 % of the total number of sperm cells recovered in the uterine horn, the UTJ, the isthmus, and the ampulla. The difference ranged from 0 to 100 % for all four segments. The difference between horns was not affected by treatments, reflux or duration of insemination. The difference between horns was neither affected by the difference in number of pre-ovulatory follicles on the ovaries.

Duration of insemination and reflux of semen. The time needed for infusing the 40 ml of treatment solution, varied greatly between sows, and was lowest for the clenbuterol treatment ($P < .10$): median time was 25 sec (7 to 60 sec), 58 sec (15 to 115 sec),

Table 2. Number of sperm cells (x 1000) recovered from the different parts of the genital tract of sows, after treatment with clenbuterol, saline (control), or cloprostenol, ten minutes before insemination. Figures are expressed absolutely and as percentage of the number recovered from the uterine horn.

	CLENBUTEROL (N = 9)		CONTROL (N = 9)		CLOPROSTENOL (N = 8)	
	Median	Range	Median	Range	Median	Range
Segment			Absolute numbers (× 1000)			
Ampulla	2.592	0 - 91.2	3.549	0 - 20.3	2.257	0 - 6.7
Isthmus	3.152^{x}	0.4 - 10	2.602^{x,y}	0 - 23.2	1.008^{y}	0.1 - 14
Oviduct	6.990	1.5 - 101.2	10.621	0 - 26.7	4.709	0 - 19.2
UTJ	896.007^{a}	147 - 2250	557.615^{a}	18 - 1565	78.275^{b}	9 - 2065
Uterine horn	1598.406^{a}	252 - 19210	290.521^{a}	3 - 9088	40.572^{b}	0 - 1334
			Relative to numbers in uterus (%)			
Ampulla	0.1	0 - 28.3	0.4	0 - 7.0	2.3	0 - 193
Isthmus	0.2	0.01 - 3.7	0.3	0 - 8.2	0.5	0 - 24
Oviduct	0.4^{x}	0.02 - 31.3	0.6^{x}	0 - 8.6	8.0^{y}	0.3 - 193
UTJ	43^{a}	7.5 - 368	134^{b}	3.4 - 1743	201^{b}	7.1 - 932

^{a,b} Figures with different superscripts indicate significant differences ($P < .05$) between treatments. ^{x,y} Figures with different superscripts indicate significant differences ($P < .10$) between treatments.

and 64 sec (14 to 240 sec) for the clenbuterol, control and the cloprostenol treatment. The time needed for infusing the semen dose did not differ between treatments: median time was 21 sec (10 to 63 sec), 58 sec (20 to 100 sec), and 59 sec (10 to 675 sec), respectively. Within sows the time needed for infusing the semen was equal to the time needed for infusing the hormone for the control and the clenbuterol groups. In contrast, cloprostenol increased the time needed for insemination (+ 46 sec on average), but the effect was not significant. During insemination, hardly any reflux was recorded; in only 4 of the 26 sows a high amount of reflux was recorded, distributed evenly among treatments.

Fertilisation

Oestrus and ovulation. For 55 of the 60 sows used for embryo recovery, ovulation took place 40 h (34 - 46 h) after GnRH on average. Four sows had ovulated before the first time ultrasound detection was performed, and one sow did not ovulate at all. Two sows were found to have only one uterine horn at slaughter. Further analyses were based on the remaining 53 sows. Forty-five of the 53 sows exhibited behavioural oestrus. The sows exhibiting oestrus came into oestrus between 25 h before and 23 h after insemination; the interval from the onset of oestrus to insemination was 5 ± 2 h on average. The interval from weaning to oestrus (91 ± 2 h), the duration of oestrus (50 ± 2 h), and the timing of ovulation were not affected by the treatments.

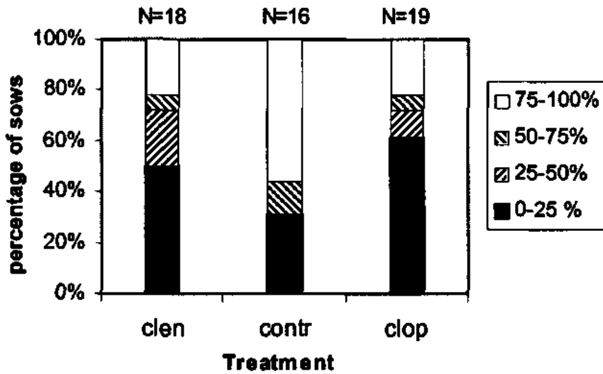

Figure 1. Percentage of sows with either 0-25 %, 25-50 %, 50-75 %, or 75-100 % of their oocytes fertilised, after intra-uterine infusion with clenbuterol (clen), saline (contr), or cloprostenol (clop), ten minutes before insemination.

Fertilisation. The mean number of corpora lutea 5 days after ovulation was 20.7 ± 0.4, and the average recovery of embryos and oocytes was 86 % of the number of corpora lutea. The fertilisation rate varied from 0 to 100 %, and was distributed dichotomously (Figure 1); 40 % of the sows had a fertilisation rate of less than 20 %, and 32 % of the sows had a fertilisation rate of more than 80 %. Degenerated embryos were observed in 18 of the 53 sows. In these sows, 1.7 (1 to 4) degenerated embryos were

observed on average. Fertilisation rate was reduced ($P < 0.05$) by both the clenbuterol and the cloprostenol treatment, compared to the control treatment (Table 3). The median number of accessory sperm cells was also reduced by the two treatments ($P < 0.05$): the number of sperm cells was 2.1 per embryo for the control group vs. 0.6 and 0.8 per normal embryo for the clenbuterol and the cloprostenol treatment (Table 3). The number of accessory sperm cells was related to the fertilisation rate. In case the sow average was < 2 sperm cells per embryo, the fertilisation rate varied between 0 and 100 %, whereas in case the sow average was $\geq$ 2 sperm cells per embryo, fertilisation rate was $\geq$ 80 %. In the control group there were relatively more sows with > 2 sperm cells per embryo ($P < 0.10$).

Table 3. Fertilization and embryo parameters for sows treated with clenbuterol, saline (control), or cloprostenol before insemination.

	CLENBUTEROL			CONTROL			CLOPROSTENOL		
		SD[1]	Range		SD	Range		SD	Range
Median Fertilization[2]	26[a]		0 - 100	81[b]		0 - 100	21[a]		0 - 100
Median Accessory sperm cells[2]	0.6[a]		0 - 38	2.1[b]		1 - 21	0.8[a]		0 - 19
Number of nuclei	27	13	6 - 48	36	15	15 - 61	28	14	7 - 53
Cell cycles	4.4	0.9	2.4 - 5.5	4.9	0.7	3.8 - 5.9	4.4	0.9	2.5 - 5.7

[1]SD: Standard Deviation. [2]For figures presented as medians, standard deviation is not given, because distributions were skewed.

The mean number of nuclei per embryo and the number of cell cycles were 27 (SD = 14) and 4.4 (SD = 0.9) for clenbuterol, 36 (SD = 15) and 4.9 (SD = 0.7) for the control group, and 28 (SD = 14) and 4.4 (SD = 0.9) for the cloprostenol treatment. The differences between treatments were not significant. A higher fertilisation rate was associated with a higher number of nuclei ($r = 0.56$; $P < 0.01$).

Duration of insemination and reflux of semen. The time needed for infusing the 40 ml of treatment solutions varied greatly between the sows inseminated for recovery of embryos, but did not differ between treatments: median time was 40 sec (4 to 330

Table 4. Number of sows with either 0-50 % or 50-100 % of their oocytes fertilised, in relation to the amount of backflow observed during insemination, after treatment with clenbuterol, saline (control), or cloprostenol before insemination.

	CLENBUTEROL Fertilization (%)		CONTROL Fertilization (%)		CLOPROSTENOL Fertilization (%)		TOTAL[1] Fertilization (%)	
Amount of Backflow	$\leq$50	>50	$\leq$50	>50	$\leq$50	>50	$\leq$50	>50
No	12	4	2	9	2	1	16	14
Small	1	1	1	2	6	3	8	6
High[2]	0	0	2	0	5	1	7	1

[1]A high amount of backflow was related to a fertilization rate <50 % (χ^2-analysis; P < .10).
[2]For the cloprostenol treatment, more sows had a high amount of backflow (χ^2-analysis; P < .05).

sec), 30 sec (5 to 210 sec), and 33 sec (9 to 510 sec) for the clenbuterol, control and the cloprostenol treatments. The time needed for infusing the semen dose was lowest for the clenbuterol group and highest for the cloprostenol group (P < 0.05): median time was 20 sec (5 to 210 sec), 52 sec (10 to 218 sec), and 97 sec (12 to 375 sec), for the clenbuterol, control and the cloprostenol treatment. Within sows, the time needed for infusing the semen was equal to the time needed for infusing the hormone, for the control group. Clenbuterol, however, decreased the time (-23 sec) needed for infusing the semen compared to the time needed to infuse the hormone, within sows. Cloprostenol, in contrast, increased the time (+29 sec) needed for infusing the semen compared to the time needed to infuse the hormone, within sows. These differences between the treatments, however, were not significant. Reflux of semen during insemination was increased by the cloprostenol treatment (P < 0.05): 6 of the 18 cloprostenol sows had a high amount of reflux, compared to 0 of the 18 clenbuterol sows, and 2 of the 16 control sows (Table 4). Furthermore, a high amount of reflux was related to a poor fertilisation rate (P < 0.10): 7 of the 8 (88 %) sows with a high amount of reflux had a fertilisation rate lower than 50 %, whereas 24 of the 44 (55 %) sows with little or no reflux had a fertilisation rate lower than 50 %.

Discussion

For the control sows, the fertilisation rate was in a normal range for sows inseminated with a low dose around 30 h before ovulation (Soede et al., 1995; Steverink et al., 1997). Timing of insemination and sperm cell dose (0.5 billion) in the current experiment were aimed at achieving intermediate fertilisation results. In that way, both positive and negative effects of treatment on fertilisation rate could be detected. Both suppression and stimulation of uterine contractility adversely affected fertilisation in the present study. Such a low fertilsation rate is most probably due to a decreased number of sperm cells being present in the oviduct around the time of fertilisation, which was reflrected in the low number of accessory sperm cells in the two treatments. Saacke (1998) already suggested that the number of accessory sperm cells can be regarded as a measure of the number of fertile sperm cells present around the time of fertilisation. Embryonic development was not affected by the treatments. The mean number of cell cycles was higher for the control treatment, but this was related to the higher fertilisation rate in this group. Embryonic development is normally related to the fertilisation rate (Kemp & Soede 1997), a lower fertilisation rate correlating with a lower number of cell cycles.

The number of sperm cells in the samples from the oviducts were low, and therefore sperm cell count was less reliable in these samples. Besides, with the applied method, recovery of sperm cells from the oviducts varied around 80 % of the sperm cells. Both aspects probably contributed to the variation between samples of the oviducts. Nevertheless, the method applied in this study is an extension of methods described earlier by Mburu et al. (1996), and by Matthijs et al. (2000), and was regarded to be suitable to study sperm transport through the genital tract.

Sperm transport to the site of fertilisation is believed to be a combination of both *passive* and *active* transport. Passive transport is more important in the initial phase of sperm transport, i.e. from the site of deposition (cervix) to the proximal uterus and the utero-tubal junction (UTJ) (Scott, 2000). The passive part of sperm transport is probably due to the flow of fluid caused by gravity and by contractile movement of the uterine horns, and requires a minimal volume (ca 50 ml) of the inseminate during AI

(Baker, 1969). From the cervix, the uterine horns progress downwards into the abdominal cavity, and then upwards again, towards the oviducts and ovaries. Contractile activity probably promotes distribution of sperm-containing fluids throughout the whole tract, even enabling unilaterally, deep intra-uterine deposited semen to be distributed over two horns (Rath, 2000).

The flow of the inseminate into the genital tract during insemination, being the first part of *passive transport*, was fastest for the clenbuterol treatment, and slowest for the cloprostenol treatment, as based on the duration of insemination. Suppression of contractility by clenbuterol treatment probably enabled flow of the semen due to gravital force, without being obstructed by phasic contractions of the uterine horns. In contrast, stimulation of contractility by the cloprostenol treatment prolonged the time needed for the semen to flow into the genital tract, probably because the uterine horns were periodically obstructed by contractions during insemination. In a previous study, the cloprostenol treatment increased the frequency of contractions from 11 to 22 h^{-1}, and the amplitude from 44 to 55 mmHg (Langendijk et al, unpublished). In the same study, a clenbuterol dose of 0.30 mg reduced frequency of contractions from 18 to 6 h^{-1}, and amplitude from 57 to 47 mmHg. In the current study, reflux of semen was increased by the cloprostenol treatment. Uterine contractility was probably stimulated by cloprostenol to such an extent, that uptake of semen by the uterine horns was impeded, thus limiting the number of sperm cells reaching the proximal part of the uterine horn. This was confirmed both by the low fertilisation rate and the low number of accessory sperm cells in sows killed at 5 d after insemination, and by the low number of sperm cells in the proximal uterus and UTJ of sows killed on the day of insemination. Steverink et al. (1998) also found that a high amount of semen reflux during insemination with a low sperm dose (< 1 billion) is related to reduced fertilisation rates.

Active sperm transport, i.e. transport due to intrinsic movement of sperm cells, is probably important for migration of sperm cells from the proximal uterus into the UTJ and the oviduct. In the hours following insemination, the number of sperm cells in the uterine horns decrease rapidly, due to semen reflux, phagocytosis, and transport into the oviducts. In this period the number of sperm cells in the UTJ and oviduct increase

(First et al., 1968; Pursel et al., 1978; Viring, 1980). The UTJ and the lower part of the isthmus are considered to be sperm reservoirs, acting as a storage place for sperm cells, until around the time of ovulation. The sperm reservoir forms a barrier to sperm cells, because of its morphology, the consistency of the luminal mucus, oviductal motility, sperm-epithelial adhesion, and ciliary movements of oviductal cells (Rodriguez-Martinez, 2000). This barrier is selective, it favors passage of live and motile sperm cells (Baker and Degen, 1972). Around the time of ovulation, changes in the utero-tubal and oviductal environment occur, which promote the ascent of sperm cells towards the ampulla and the site of fertilisation (Hunter, 1981).

Although for clenbuterol the number of sperm cells in the proximal uterus was equal to or higher than the other treatments, the number of sperm cells in the UTJ was relatively lower compared to both the other two treatments, and the number of sperm cells in the oviduct was relatively lower than for cloprostenol. Apparently, of the sperm cells that reached the proximal end of the uterine horns, relatively less were able to pass through the UTJ into the oviduct (*active transport*) for the clenbuterol treatment. Capacity of the UTJ and isthmus was not limiting, because for all treatments, the number of sperm cells in the UTJ and isthmus was linearily related to the number in the proximal uterus (data not shown). Moreover, other studies have found a larger number of sperm cells in the UTJ and isthmus (First et al., 1968; Viring, 1980). This means that the clenbuterol treatment either reduced the ability of the sperm cells to penetrate the UTJ and the oviducts, or affected the environment of the UTJ and the oviducts, rendering them less accessible to the passage of sperm cells, or both. The reduction of contractility by the clenbuterol treatment might have prolonged the time needed for transporting the sperm cells from the distal part to the proximal part of the uterine horns (*passive transport*). The lifespan of sperm cells is much shorter in uteri than in the oviducts (First, 1968; Scott, 2000). The increased transport time to the proximal uterus might thus have affected the ability of the sperm cells to penetrate the UTJ and the oviducts. Besides affecting the intrinsic quality of the sperm cells by increasing transport time, uterine contractions may also play a role in the propulsion of sperm cells from the uterine horns into the oviducts. Baker & Degen (1972) observed a pulsatile appearence of fluid from the uterine horns into cannulated oviducts shortly after insemination. The reduction of contractility by the clenbuterol treatment might

have reduced this passive transport into the UTJ and isthmus. Accessability of the oviducts might also have been affected by the clenbuterol treatment. As a β-adrenergic agonist, clenbuterol might have reduced oviductal contractility on one hand, probably relaxing the oviductal barrier. On the other hand, clenbuterol might have dilated blood capillaries around the UTJ, thus increasing swelling of the tissue of the UTJ, and reducing accessibility. Oviduct status was not recorded in this study, but for these hypothesized effects of the treatments on the oviduct to occur, plasma levels of clenbuterol and cloprostenol should have been high enough until around the time of sperm cell recovery. Cloprostenol has a relatively short half-life of around 2 h (R. Kirkwood, personal communication), which would be too short to affect oviductal status around fertilisation or around the time of sperm cell recovery. It is not clear whether plasma levels of clenbuterol around the time of colonization of the sperm reservoirs was high enough to affect contractility of the uterus and the oviducts. Absorption of clenbuterol from the uterine horns, based on oral administration in other species (Smith, 1998), is probably completed within ca 2 h, indicating a fast decline in the local concentration of clenbuterol around the uterus. The plasma half-life time of clenbuterol, however, ranges from 9 h in rabbits to 30 h in humans (Yamamoto et al., 1985).

In summary, a minimum level of uterine contractility around insemination seems to be important for swift transport of sperm cells to the tubal end of the uterine horns. A prolonged phase of transport through the uterine horns might adversily affect the ability of sperm cells to penetrate the UTJ and the oviduct, thus reducing the number of fertile sperm cells around the time of fertilisation. However, above a certain level, uterine contractility increases reflux of semen to such an extent, that number of sperm cells reaching the tubal end of the uterine horns are reduced. More research has to be conducted to establish an optimal level of uterine contractility and to investigate the importance of the timing of uterine contractility relative to the moment of insemination.

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GENERAL DISCUSSION

I Boar stimuli and estrous behavior

Introduction

In most mammalian females, the period around ovulation is marked by estrous behavior. In sows, estrus is normally defined as the period during which sows show receptive behavior, i.e. a 'standing reflex', in reaction to certain stimuli. These stimuli are one or more stimuli which are involved in the courtship behavior of the boar and in the act of mating, and can be olfactory (boar odor), visual (presence of the boar), auditory, and tactile (rubbing of back and flanks) (Signoret, 1970). Mimicking (a combination of) such stimuli can elicit receptive behavior. Whether or not a sow will show receptive behavior in reaction to certain stimuli depends on the level, or 'potency', of such stimuli, and on the responsiveness of the sow to the stimuli. The detection of receptive behavior using boar stimuli is important for estrus-based insemination strategies. To enable more accurate timing of inseminations, the prediction of the time of ovulation is also important. Estrous behavior or the change in estrous behavior might be related to the time of ovulation. In the first part of the General Discussion, the influence of boar stimuli on estrous behavior, and the relation with the time of ovulation are discussed.

Boar stimuli and the induction of receptive behavior

Detection of estrous behavior

During estrus, the responsiveness to stimuli used to evoke receptive behavior first increases, and then decreases again. As a consequence, during estrus different phases can be distinguished, depending on the 'level' of the stimulus needed to evoke receptive behavior. Willemse & Boender (1967) for example, distinguished an 'inseminator period', during which an inseminator could evoke a standing reflex by mimicking tactile boar stimuli (BPT: Back Pressure Test, manual stimulation of the back and flanks of a sow). This period covered the middle two-thirds of the 'boar period', the period during which physical contact with a boar would evoke a standing reflex.

The inseminator, therefore, is less potent in evoking estrous behavior than a boar, and consequently, the duration of estrus recorded by an inseminator will be shorter than when using a boar. Several studies have focused on the potency of different stimuli or combinations of stimuli to evoke receptive behavior, and as such, on their use in the detection of estrus. A BPT in combination with boar odor or components of boar odor has been shown to evoke a standing reflex in a percentage of estrous sows (up to 60%) that do not show a standing reflex in response to a BPT alone (Signoret & Bariteau, 1975; Reed et al., 1974; Perry et al., 1980). The importance of boar odor also appears from the fact that boars without submaxillary salivary glands are incapable of evoking receptive behavior in estrous gilts (Perry et al., 1980). Auditory stimuli can also evoke a standing response in about 50% of sows that do not show a standing response to a BPT alone. Combination of auditory and olfactory stimuli can induce a standing response in up to 90% of such sows (Signoret, 1970). The data in chapter 5 of this thesis show that a BPT, alone or in combination with boar odor aerosol, elicits a standing reflex in not more than one third of the sows, both on Day 1 and on Day 2 of estrus. The variability between sows in the stage of estrus probably contributed to the observed differences in potency between levels of boar stimuli in the studies mentioned above. However, some sows remain insensitive to certain stimuli throughout the entire period of estrus. As described in Chapter 1 for example, a BPT did not evoke a standing reflex at any occasion during estrus in about 30% of the sows. Apparently, components of boar stimuli are less potent in inducing a standing relex than a 'whole' boar, and combination of different components of boar stimuli increases the chance of inducing a standing response in estrous sows.

From the above it appears that in terms of detection of estrus, the use of a boar seems to reduce the risk of 'missing' estrous sows to a minimum. The use of components of boar stimuli increases the risk of missing estrous sows, because they are less potent in evoking estrous behavior, and because the duration of estrus recorded with such stimuli is shorter. The question rises whether there is a combination of stimuli which is even more effective than the presence of a boar. Hemsworth & Hansen (1990) designed a Detection-Mating-Area (DMA), which consists of an open space surrounded by several boar pens. Stimulation of estrous behavior could be expected to be more intense in such a DMA. In the present work (Chapter 1), the use of a DMA was

compared to detection of estrus with a boar and with a BPT alone, for the same group of sows. The duration of estrus for these sows recorded by BPT was 24 h, by BPT in presence of the boar 45 h, and by BPT in a DMA 55 h ($P < .05$). Moreover, the percentage of sows detected in estrus was higher in the presence of a boar or in the DMA (88% and 94%, respectively), compared to detection by BPT alone (41%). However, for a second group of sows that were not tested in the DMA, the duration of estrus recorded by BPT in the presence of a boar (52 h) did not differ from that recorded for the other group of sows in the DMA. Moreover, the percentage of sows detected in estrus by BPT was higher (68% vs 41%) for the group that was not tested in the DMA. Apparently, the use of extra stimuli (DMA in the first group) compared to the presence of a boar (first group) did not increase the chance of evoking receptive behavior; the sows adapted their responsiveness to the highest level of boar stimuli they were confronted with. Adaptation, or habituation to boar stimuli was also described by Hemsworth & Hansen (1990) and Dyck (1988), who both found that continuous housing adjacent to the boar reduced the duration of estrus in sows after weaning.

This type of adaptation does not necessarily occur at a lower level of stimuli: in Chapter 3, a study is described comparing detection of estrus with a BPT in a group of sows without any boar contact, to detection of estrus in the presence of a boar in a different group of sows. The duration of estrus as recorded in the presence of a boar (56 h) was longer than that recorded by BPT (38 h) in the same group of sows before introduction of the boar, but also longer than that recorded by BPT (39 h) in the group of sows that had no boar contact. Apparently, the presence of one boar during estrus detection did not affect responsiveness to lower levels of stimuli (BPT) and was more effective in eliciting receptive behavior than the BPT. It is an interesting question whether this also applies when other combinations of boar stimuli are compared to a BPT in the presence of a boar. A BPT in combination with boar odor (Chapter 5) for example, was shown to be less potent than the presence of a boar in evoking receptive behavior, in the same group of sows. In the scope of this thesis, however, boar odor was not tested in a group of sows that did not experience boar contact.

Summarising, high levels of boar stimuli used to detect receptive behavior cause adaptation of the sows' responsiveness to those levels and reduce responsiveness to lower levels of stimuli. At lower levels of stimuli (BPT), the responsiveness of sows does not adapt to the stimuli applied, creating a risk of missing estrous sows. Therefore, with detection of estrus three times a day, the presence of a boar seems necessary to eliminate such a risk. The use of higher levels of stimuli, such as a DMA, does not seem to add to the detection of estrus.

Regulation of receptive behavior

To date, it is not clear how the display of receptive behavior is regulated neuro-endocrinologically. On a long-term basis, the display of receptive behavior in part depends on the exposure to elevated levels of estrogens (Signoret, 1967; Dial et al., 1983). In ovariectomised sows, treatment with estrogens causes behavioral estrus, and the duration of estrus is correlated with the dosage of estrogens administered. Soede et al. (1997) showed that in spontaneously ovulating sows, the duration of estrus was not related to the level of circulating estrogens, indicating that other factors are also important. The level of stereotype behavior in these sows was inversely related to the duration of estrus. Esbenshade & Huff (1989) found that treatment with morphine, an endogenous opiate, shortened the duration of estrus in gilts from 38 h to 21 h. What determines the onset and the termination of estrus is not clear. The pre-ovulatory LH surge occurs around the onset of estrus (Soede, 1997; van de Wiel, 1981), but is not necessary for the onset of estrus, as sows with a longer duration of estrus already show receptive behavior before elevation of plasma LH levels. Probably, estrogens cause a certain maturation of the neuroendocrine system needed to express receptive behavior. As a consequence, this system becomes sensitive to boar related stimuli, which can then induce a neuroendocrine cascade of events, with the display of the standing response as one of the exponents.

Little is known about the events triggered by boar-related stimuli that lead to a standing reflex. Krzymowoski et al. (1999) demonstrated a humoral pathway for the uptake of boar pheromones from the nasal cavity and transfer to the pituitary and the rest of the brain. Spraying 5α-androstenon (a component of boar odor) in front of the

nose of a sow, can induce the release of hypophyseal oxytocin during estrus (Mattioli et al., 1986). Oxytocin release is also observed during mating (Claus & Schams, 1990; Kotwica et al., 1995) and during fenceline contact with boars (Chapter 5). Prolactin is also released upon exposure to a boar, in the same surgelike fashion as oxytocin (Prunier et al., 1987; Kotwica et al., 1995). Treatment of gilts with naloxon, an endogenous opiate inhibitor, increases the initial peak in oxytocin release shortly after presentation to the boar. Inhibition of prostaglandin synthesis, on the other hand, reduces the release of both prolactin and oxytocin during mating, as well as the time that the animals are willing to show receptive behavior (Kotwica et al., 1995). Prostaglandin released by the uterus interacts with the release of oxytocin and prolactin. Apparently, both oxytocin and prolactin are involved in the display of receptive behavior, and modulation of the secretion of these neuropeptides can affect receptive behavior. However, oxytocin release is not necessary to display receptive behavior. In Chapter 5, a BPT alone or in combination with boar odor aerosol, did not cause a release of oxytocin in any sow, but induced a standing reflex in one third of the sows.

However, oxytocin might have a facilitatory role in the exhibition of receptive behavior. Data from Chapter 5 of this thesis indicate a positive relationship between the magnitude of oxytocin release after introduction of a boar and the time receptive behavior is exhibited: below 75 pg/ml the duration of the standing reflex varied between 0.5 and 5 min whereas above 75 pg/ml the duration was at least 4 min. What triggers a standing reflex in the absence of oxytocin release is still not clear. More research is needed to elucidate such factors, and factors involved in the release of oxytocin.

Receptive behavior and the timing of ovulation

The period of estrus is an indication of the time of ovulation, which is important for the timing of insemination. With estrus detected in the presence of a boar, ovulation takes place at a fairly constant two thirds of the way through the period of estrus. Because the duration of estrus varies a lot, the onset of estrus is not an accurate predictor of ovulation (for review, see Soede & Kemp, 1997). The data described in Chapter 2 of

this thesis show that when using other stimuli to detect estrus, the timing of ovulation still shows too much variation relative to the onset of estrus to accurately predict ovulation. With different stimuli, however, the period of estrus can be divided into different phases. Using a BPT receptive behavior was first detected 24 h before ovulation, using a BPT in the presence of a boar 34 h before ovulation and, using a BPT in a DMA (area surrounded by four boars) receptive behavior was first detected 41 h before ovulation (Chapter 2). The time between these phases explained some of the variation in the time of ovulation relative to the onset of these phases, but not enough to accurately predict ovulation. Soede et al. (1996) also found a weak relationship between the duration of estrus detected with a BPT and the duration of estrus detected with a BPT in the presence of a boar ($R^2 = 0.25$). Apparently, the change in reponsiveness to boar stimuli does not follow the same pattern for all sows.

Summarising, the time of ovulation shows too much variation relative to the onset of estrus to use 'onset of estrus' as a predictor for ovulation. Using other stimuli than a conventional BPT in the presence of a boar to detect estrous behavior does not reduce this variation. The time between different phases of estrus, as defined by using different stimuli to detect receptive behavior, is related to the timing of ovulation, but not strong enough to improve the prediction of ovulation.

The influence of boar stimuli on follicular development leading to estrus

Although this was not a major aim of this thesis, it was also found that boar stimuli are also important for inducing the onset of ovarian activity. Boar stimuli can advance estrus in weaned sows by days, indicating an effect on the onset or dynamics of ovarian activity (Walton, 1986; Pearce & Pearce, 1992). In an experiment described in Chapter 3 of this thesis, only 30% of primiparous sows that had no contact with a boar ovulated within 9 days after weaning. During the first days after weaning all sows showed follicular development, but apparently a number of sows was not able to maintain follicular growth through to ovulatory size. A reason for this might be the metabolic constraints suffered by these animals. Sows that did not ovulate weighed less and had less back fat at weaning than sows that did ovulate within 9 days after weaning. Boar contact increased the percentage of sows ovulating in this period to

51%. Apparently, boar stimuli could overcome the metabolic constraints in some of the sows, and sustain follicular growth towards ovulation. Boar contact commenced on the third day post weaning. Earlier contact with a boar might have prevented the delay in the onset of estrus that was observed in the extra sows that ovulated in the group with boar contact. Boar contact during lactation has been shown to reduce the interval to estrus after weaning (Petchey & English, 1980; Henderson & Hughes, 1984; Newton et al., 1987)

Boar stimuli most likely act through neuro-endocrine pathways that are involved in the secretion of LH by the pituitary, and thus the regulation of ovarian activity. In a 'normal' situation, sows come into estrus 4 to 7 days after weaning. A higher secretion of LH at the day of weaning results in a shorter weaning-to-estrus interval (Shaw & Foxcroft, 1985; van den Brand, 2000). Follicle growth in later stages of the follicular phase is also dependent on LH pulsatility (Driancourt et al., 1995; Guthrie et al., 1990). There are situations where LH secretion might be limiting for the onset of ovarian activity, in which boar stimuli might be helpful. In sows that had been anestrus for 21 days, Van de Wiel et al (1993) found an increase in LH pulsatility when the sows were introduced to a boar. Further, gilts that showed an increase in LH pulsatility immediately after introduction to a boar, came in estrus within 2 months after introduction to the boar, in contrast to those gilts that did not show an increase in LH pulsatility after boar introduction (Kingsbury & Rawlings, 1993). In a parallel group of gilts without boar exposure, no increase in LH pulsatility was observed, indicating a boar effect on LH pulsatility. Therefore, in the experiment with primiparous sows described in Chapter 3, the contact with a boar after weaning might have increased LH pulsatility to a level sufficient to stimulate follicular growth towards ovulation, in sows that otherwise would not have ovulated within 9 days.

Summarising, in sows with limited follicular development, boar contact can restore follicular development towards ovulation in a part of the sows. Stimulation of LH release by boar contact might be the factor explaining this effect. With boar contact commencing on the day of weaning or even earlier, the delay in the onset of estrus in these sows might be prevented, and the number of sows in which follicular growth is sustained might be increased.

II Boar stimuli, uterine activity and sperm transport

Introduction

During mating and artificial insemination in sows, semen is deposited intra-cervically. However, due to the volume, the bulk of semen is flushed directly into the lumen of the uterine body immediately post-cervically. Therefore, semen depostion is often referred to as intra-uterine. From the site of deposition, sperm cells have to be distributed over both horns and to be transported to the tubal end of the horns. There, a relatively small number populates the utero-tubal junction and the first part of the oviduct, which serve as a sperm reservoir. The transport of sperm cells through the uterine horns is believed to be a passive process, in which intrinsic sperm cell motility takes no part. The passive process probably depends on the flow of sperm cell-containing fluids in the uterine lumen, driven by gravitational force and uterine contractility. In the scope of this thesis, the role of uterine activity in the transport of sperm cells through the uterine horns will be discussed.

Spontaneous uterine activity

Uterine activity around estrus in sows has been studied using several *surgical* methods (Table 1), either by applying strain gauges on the uterine wall to record stretch of the uterine musculature (Bower, 1974; Scheerboom, 1987), or by applying electrodes to record electromyographic activity (Zerobin and Sporri, 1972; Taverne, 1982; Brussow, 1988; Claus, 1989). A drawback of both strain gauges and electromyography is that these methods do not directly record pressure changes in the uterine lumen, since electrical activity does not necessarily induce muscular contraction. This translation depends on the sensitivity of the myometrium to electrical triggering, which in turn depends on the physiological status of the myometrium (Scheerboom, 1987). Several types of electrical activity, as defined by Scheerboom (1987), all coincided with myometrial stretch as recorded with strain gauges. However, it was not clear from the study by Scheerboom (1987) how electrical activity correlates quantitatively with myometrial contraction. Intraluminal pressure changes are a more direct indication of

Table 1 Myometrial activity during and around estrus, as reported by different authors, using different methods.

Author & Method	Parameter	Diestrus	Proestrus	Estrus
Scheerboom (1987)	Frequency of MC[1]	4 h^{-1}	14 h^{-1}	10 h^{-1}
EMG[2]	Amplitude	115 μV	227 μV	870 μV
Claus et al. (1989)	Frequency of bursts[3]	90 h^{-1}	17 h^{-1}	20 h^{-1}
EMG	Duration of bursts	2 sec	22 sec	32 sec
	Amplitude	180 μV	1720 μV	2040 μV
	Luteal phase activity below detection level			
Brussow et al. (1988)	Frequency of AP[4]		8 h^{-1}	29 h^{-1}
EMG	Relative duration[5]		22 %	25 %
Bower (1974)	Frequency of contractions	0 h^{-1}	8 h^{-1}	15 h^{-1}
Strain gauges	Force of stretch	0 gm	14 gm	15 gm
Zerobin & Sporri (1972)[6]	Frequency of contractions		~ 60 h^{-1}	21 – 30 h^{-1}
Intraluminal pressure	Amplitude of contractions	< 8 mmHg	< 15 mmHg	35 – 45 mmHg
	Duration of contractions		~ 25 sec	max 60 sec
Langendijk et al. (2001)	Frequency of contractions		15 h^{-1}	22 h^{-1}
Intraluminal pressure	Amplitude of contractions		20 mmHg	40 mmHg
	Duration of contractions		60 sec	60 sec

[1]MC = Myoelectrical complex; [2]EMG = Electro Myography; [3]bursts: bursts of electrical activity; [4]AP = action potentials; [5]relative duration: duration of electrical activity, relative to measuring time;[6]For Zerobin and Sporri approximate figures are given, because of lack of quantitative information.

muscular contraction, especially in terms of transport of sperm-containing fluids. Döcke and Worch (1963) recorded uterine intraluminal pressure using a fluid filled balloon inserted into the cervical end of the uterus. There are no other reports on the use of *non-surgical* techniques to record intraluminal pressure in sows. Recently, we developed a non-surgical technique (Chapter 4) by adapting a device developed for

non-surgical embryo-transfer in pigs (Hazeleger & Kemp, 1994). With this device it is possible to pass the cervix and introduce a fluid-filled catheter into the distal end of the uterine horns, to record intraluminal uterine pressure. With this technique, spontaneous myometrial activity around estrus was studied.

Two to four days before estrus, myometrial activity is low in terms of frequency and amplitude of contractions (Chapter 4). A percentage of sows (around 50%) does not show any contractility at all around this time, and the sows that do show contractility tend to have a low frequency and amplitude of contractions, compared to the situation during estrus (Table 1). Approaching estrus, the percentage of sows showing myometrial activity increases, and the frequency and amplitude of contractions increases too. During estrus, all sows show myometrial activity, and frequency and amplitude of contractions are maximal, and after estrus, uterine activity decreases again. Using the non-surgical technique, data could be collected in a relatively large number of sows (Chapter 4), which enabled us to study variation between sows. Variation between sows during estrus is huge: frequency of contractions ranges from 6 to 40 h^{-1}, and amplitude of contractions ranges from 16 to 57 mmHg. Nevertheless, the difference between sows is consistent over the days around estrus, i.e. sows with a relatively high level of uterine activity during the days before estrus also show a relatively high level of uterine activity during estrus. This indicates sow-dependent variation. Factors that contribute to variation in myometrial activity are discussed further on.

Cyclical variation in uterine activity, that is an increase in uterine activity during estrus, was also observed by other authors. Force of contraction increases during estrus, regardless what method is used (Zerobin and Sporri, 1972; Bower, 1974; Scheerboom, 1987; Brussow, 1988; Claus et al., 1989), but not all authors report an increase in the frequency of contractions during estrus (Table 1). However, quantitative comparisons between studies are difficult because site of recording, the method of recording (electrical activity, muscular stretch, intraluminal pressure), and the definition of parameters differ between studies. With electromyographic observations sometimes a lower frequency of electrical activity is reprorted during estrus than outside estrus (Claus et al., 1989; Scheerboom, 1987). However, the lower frequency of electrical

activity during estrus does not necessarily correlate with lower contractile activity. During estrus, amplitude and duration of electrical bursts are more pronounced (Claus et al., 1989), and the myometrium is more sensitive to electrical input. Thus, a lower frequency of electrical burst during estrus can still result in a lower frequency of contractions. Nevertheless, Zerobin & Sporri (1972) also observed a decrease in the frequency of contractions during estrus, and they used intraluminal catheters. However, the contractions during estrus were of much higher amplitude (35 to 45 mmHg) compared to the contractions outside estrus (up to 3 mmHg), and with different criteria probably a lower frequency of contractions would have been observed outside estrus. Concluding, during estrus the amplitude of contractions is increased, and the frequency of contractions very much depends on the criteria used to define contractility. However, 'functional' uterine activity, in terms of sperm transport, is probably increased during estrus.

The variation in myometrial activity throughout the estrous cycle and between sows mainly depends on levels of estrogen and progesterone. Progesterone inhibits, and estrogen increases uterine activity. From other species than the pig it appears that estrogen increases gap junctional coupling between myometrial cells (Verhoeff et al., 1986), myosin content of myometrial cells (Michael et al., 1969), and pace-maker activity of myometrial cells (Finn & Porter, 1975). In swine, there is little evidence on the action of estrogens and progesteron around estrus on the myometrium. Data from Hoang-Vu et al. (1987) indicate that estrogen levels (Figure 1), but also the ratio of estrogen/progesterone are related to the duration and amplitude of electrical bursts in myometrial cells. However, in their study the levels of estrogen and progesterone might merely reflect the stage of the cycle, without proving the role of estrogens and progesterone. In the study described in Chapter 4, we observed that sows with a longer duration of estrus (3 vs 2 days) maintained the high level of uterine activity during estrus for a longer period of time. On the second day of estrus these sows had a higher level of uterine activity than sows with a shorter duration of estrus; in the latter sows uterine activity had already started to decline. For sows with a longer duration of estrus, the decline in estrogen levels occurs later relative to the onset of estrus.

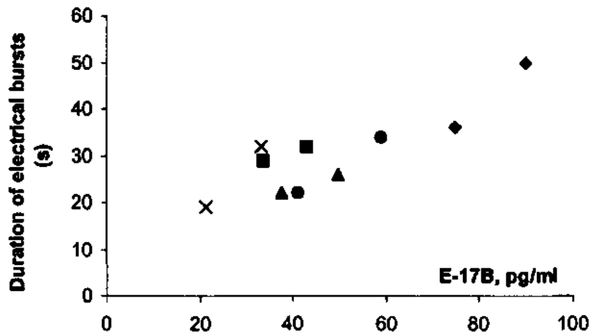

Figure 1 Plasma level of estradiol-17β on the first two days of estrus, in relation to the duration of electrical bursts recorded in an electro-myogram. Different symbols represent different sows, on Day 1 or Day 2 of estrus. After Hoang-Vu et al. (1987)

Variation between sows in observed myometrial activity might also be caused by variation within sows throughout the day. In a study with mini-pigs (Taverne et al., 1982), electrical myometrial activity showed a rythmic fluctuation throughout the day during proestrus. In a study with Landrace sows (Hoang-Vu et al., 1987), frequency of contractions was constant throughout the day during estrus. If variation throughout the day exists in sows, it might be regulated by varying levels of estrogens, oxytocin, and prostaglandins. Both oxytocin and prostaglandins are known agonists of myometrial activity. In the ewe (Gilbert et al., 1991), for example, levels of oxytocin have been shown to fluctuate during estrus. Both estradiol and oxytocin have been shown to affect the release of prostaglandin by the uterus (Edgerton et al., 2000). Fluctuation in estradiol and oxytocin might directly affect uterine activity, but also indirectly through the release of prostaglandin. Information on diurnal variation in oxytocin and prostaglandin levels in pigs during estrus is lacking. One study (Shille et al., 1979) reports low, but fluctuating levels of prostaglandin during estrus in sows. More study is needed to investigate the dynamics of these hormones and the relation with uterine activity during estrus in the pig. A more recently described candidate for regulation of uterine activity is LH. LH/hCG-receptors are present on the myometrium during estrus (Ziecik et al., 1992), and hCG has been shown to suppress myometrial

activity (Flowers et al., 1991). Whether the elevated levels of LH during the preovulatory period have a function in the regulation of myometrial activity is still not clear.

Obviously, myometrial activity is not only regulated by levels of circulating hormones, but also by the sensitivity to these hormones. Estradiol, progesterone, and oxytocin receptor concentrations (Thilander et al., 1990; Wathes et al., 1996) are known to be regulated by estradiol and progesterone. Sensitivity to oxytocin, for example, increases around estrus (Okano & Okuda, 1996; Franczak et al., 2000; sows), but can also differ between animals (Cadario et al., 1999; Gutjahr et al., 2000; mares).

Summarising, the variation in spontaneous myometrial activity around estrus is probably a reflection of altering levels of progesterone and estrogens and their receptors. Observed differences between sows might be due to sow dependent factors, like duration of estrus, but might also be caused by variation in myometrial activity throughout the day. More research is needed to establish such variation and the factors involved.

Male sexual stimuli and uterine activity

During and around mating, several sexual stimuli are involved, which might be important for their effect on uterine activity. These stimuli can be divided in *sensory stimuli*, i.e. tactile, olfactory, visual, and auditory stimuli one one hand, and *seminal plasma related stimuli* on the other hand.

The presence of the boar as a whole, has been shown to increase plasma oxytocin levels dramatically in the sow, and to increase uterine actvity as well (Claus & Schams, 1990; Chapter 5). Interestingly, in our work (Chapter 5) the increase in uterine activity was only observed in sows that had a below average frequency of uterine contractions. The same phenomenon was observed when oxytocin was injected i.m.; sows with a below average frequency of uterine contractions showed a greater increase in uterine activity. The increase in uterine activity in the presence of a boar was not related to the magnitude of oxytocin release. The difference between sows in the effect of boar

presence or oxytocin injection could have been caused by differences in the sensitivity to oxytocin due to difference in receptor concentrations, but this was not studied.

Effects of components of boar presence or mating have hardly been studied separately. *Olfactory* stimulation with 5α-androstenon can cause a release of oxytocin with the same magnitude as observed during mating, and can increase uterine activity (Mattioli, 1986; Maffeo et al., 1993). However, the effect of 5α-androstenon on oxytocin release is not unambiguous: in Chapter 5 we describe a study in which 5α-androstenon did not induce any release of oxytocin, and hardly affected myometrial activity. In that study, sows were used to regular boar contact (twice a day). Habituation to regular boar contact might reduce responsiveness of sows to components of boar stimuli, such as boar odor. Whether this is an explanation for the total lack of an olfactory effect on oxytocin release in our study is not clear. *Tactile* stimulation of the back and the flanks of a sow, in absence of a boar, does not induce oxytocin release and hardly has any effect on uterine activity (Chapter 5), even though it evokes receptive behavior. Effects of tactile stimulation of the cervix on myometrial activity has been studied by several authors. Insemination with a normal catheter, or manipulation of a catheter for uterine pressure recording through the cervix does not induce release of oxytocin (Claus & Schams, 1990; this thesis: Chapter 5). In a study by Bower (1974), sensory stimuli such as massage of the vulva and clitoris, mounting of a boar and intromission of a catheter, did not affect uterine activity. However, in his study observations continued for only about 10 min after a certain treatment, which might be too short to reliably assess effects. Zerobin (1968) found a positive effect of merely inserting an insemination catheter on uterine activity, and Pitjkanen & Prokofjev (1964) observed enhancement of uterine activity after electrical stimulation of the cervix. Insemination with a certain volume of saline or semen extender stimulates contractility (Claus et al., 1989: 100 ml; Bower, 1974: volume not described; Langendijk et al., unpublished results: 80 ml), but these effects can be contributed to both the volume of the infused fluid and to the stimulation of the cervix with the insemination pipet. Intra-uterine infusion with 80 ml of saline, through a thin intra-uterine catheter used for intraluminal pressure recording and thus avoiding cervical stimulation, does not enhance uterine activity (Chapter 4). All mentioned studies were performed using a relatively low number of sows. Taken together, however, it seems

that the insertion of an insemination catheter, and not so much the infusion of the volume associated with insemination, enhances uterine activity. Stimulation of the cervix probably stimulates uterine activity through an adrenergic or cholinergic pathway, as oxytocin is not released. Both adrenergic and cholinergic receptors have been found in the myometrium, with adrenergic receptors dominating in the longitudonal muscle layer, and cholinergic receptors dominating in the circular muscle layer (Taneike et al., 1991). Stimulation of cholinergic and α-adrenergic receptors initiates contractility, whereas stimulation of β-adrenergic receptors suppresses contractility. It is still not clear which receptor type is predominantly activated by cervical stimulation, but based on the studies mentioned above, α-adrenergic and/or cholinergic stimulation seems to dominate. The effect of excitation of the myometrium both through the release of oxytocin and through neurogenic stimulation is maintained for a considerable period of time. Uterine activity remained elevated in the period of recording (ca 1 hour) following presentation of a boar (Chapter 5) or boar odor (40 min, Maffeo et al., 1993). Hoang-Vu et al. (1987) recorded for a longer period of time and found that the effect of cervical stimulation during insemination lasted for a few hours after insemination. These stimuli are hard to compare, as they were investigated in different studies.

In contrast to the situation for sensory stimulation, there is more convincing evidence for the effect of *seminal plasma* on uterine activity. Seminal plasma has been shown to stimulate uterine motility in vitro (Einarsson & Viring, 1973). The reason for this most likely is the estrogen content of seminal plasma. A boar ejaculate can contain up to ca 12 μg of estrogens, although the level varies between boars and between seasons (Claus, 1990). Roughly half of the estrogens in an ejaculate is conjugated to the sperm cells, which likely act as carrier proteins. After insemination, the estrogens in the ejaculate cause an immediate release of prostaglandin by the endometrium (Claus, 1990). Infusion of both estrogens (Claus, 1990) and prostaglandin (Einarsson et al., 1975; present thesis, Chapter 4) have been shown to increase uterine motility in sows. The effect of infusion with a physiological dose of estrogens (11.5 μg) on uterine motility is maintained for a few hours (Claus, 1990). Mating with a boar probably results in an even more prolonged effect on uterine motility because of a more prolonged release of estrogens that are conjugated to sperm cells (Claus, 1990).

Summarising, spontaneous myometrial activity during estrus can be influenced by boar stimuli. The presence of the boar clearly induces a release of oxytocin and stimulates myometrial activity, the latter predominantly in sows with relatively low uterine activity. The effect of components of boar stimuli, such as tactile stimuli and olfactory stimuli are less clear. Cervical stimulation during insemination seems to stimulate uterine activity, but not through the release of oxytocin. Estrogens in seminal plasma have a clear effect on uterine activity, through the release of uterine activity.

Uterine activity and the concept of sperm transport

During natural mating and artificial insemination, a considerable volume (80 to 300 ml) is deposited in the genital tract. This volume is functional, because a decrease of the inseminated volume (to 20 ml) reduces fertilisation rate (Baker et al., 1968). The large volume probably serves to flush sperm cells from the cervix into the uterine horns, and to prevent the sperm cells from being retained in the cervical folds. For transport and distribution of sperm cells within the remaining part of the tract, a large volume is not necessary, since even with small volumes (less than 10 ml) of unilaterally, deep intra-uterine deposited semen, a good fertilisation rate can be achieved (Krueger & Rath, 2000; Martinez, 2001). Nevertheless, a larger volume might still accelerate the transport of sperm cells to the utero-tubal junction, thus reducing the transit time through the uterine horns. This might be important particularly when insemination takes place long before ovulation, because phagocytosis in the uterine horns reduces number of sperm cells surviving there (Woelders & Matthijs, 2001).

After semen deposition, sperm cells have to be transported to the tubal ends of the uterine horns. Within minutes after insemination, fertile sperm cells can be found in the oviducts (Baker & Degen, 1972), indicating that this process can be very fast. The first part of the uterine horns protrudes downwards from the cervix, into the abdominal cavity (see Figure 2 of the Introduction). The flow of semen into the first part of the uterine horns therefore probably depends on gravity and relaxation of the myometrium. The common assumption that semen is 'sucked' into the genital tract is probably wrong. During estrus, the luminal pressure in the uterus consists of a baseline

pressure in the relaxed state, with periodically superimposed increases in pressure due to contractions. A reduction in pressure causing suction has never been observed in our studies (Chapter 4). Moreover, stimulation of contractility increased the time needed for uptake of semen during insemination and the numbers of sows with a high amount of seminal reflux (Chapter 6). Zerobin & Sporri (1972) also observed that contractions in the caudal part of the uterus obstructed infusion of semen. Huhn et al. (1977) observed a decrease in pregnancy rate with increasing time needed for insemination. Probably, increased frequency of contractions delays the flow of semen into the first part of the genital tract. Beyond the first part, the uterine horns curl upwards and end caudally at the utero-tubal junction, near the ovaries. Transport of sperm cells through the remainder of the horns, and distribution of semen over the two horns in the case of unilateral deposition of semen, depends on uterine contractions. Additionally, transfer of sperm cells into the oviducts might in part be promoted by uterine contractions. Shortly after insemination, Baker & Degen (1972) observed fluid flowing in a pulsatile manner from a uterine cannula positioned near the oviducts. Fluid also appeared in cannulas inserted in the oviducts. Therefore, uterine contractions might contribute to the transfer of sperm cell containing fluid into the oviducts.

Although virtually all the fluid inseminated is expelled by vaginal reflux, only about 25 to 45 % of the sperm cells is lost by reflux of semen during the period of 2 to 4 hours following artificial insemination (Steverink et al., 1998; Matthijs et al., 2000). A considerable number of sperm cells is removed from the genital tract by phagocytosis. Shortly after insemination, leukocytes start to invade the uterine lumen, and the number increases up to a level of 10^7 to 10^8 per uterus, a few hours after inseminaton (Woelders, 2001; Rozeboom et al., 1998). To indicate the phagocytosing capacity of leukocytes: in an in vitro study with a ratio between leukocytes and sperm cells of 2:1, Matthijs et al (2000) found that 60 % of the sperm cells were phagocytosed in the first 15 minutes after the start of incubation. As a consequence of reflux and phagocytosis, the number of sperm cells in the uterine lumen declines exponentially after insemination. From 2 h to 24 h after insemination, Pursel et al. (1978) observed a decrease in the number of sperm cells recovered from the uterus from 0.5×10^9 to 0.5×10^6. In the sperm reservoirs, i.e. the utero-tubal junction and the first part of the

isthmus, sperm cells can survive for a much longer period of time and are relatively safe from phagocytosis, compared to the uterus (Du Mesnil du Buisson & Dauzier, 1955; Pursel et al., 1978; First et al., 1968). Additionally, sperm quality decreases more rapidly in the uterus than in the oviducts. Twenty-four hours after insemination, the motility was 0% and 30% for sperm cells recovered from the uterine horn and the oviducts, respectively (First et al., 1968). Fourteen hours after insemination, the percentage of sperm cells with normal acrosomes was 50 and 74% for the uterus and the oviduct (Pursel et al., 1978). Therefore, quick transport to the tubal end of the uterine horns probably increases the number of sperm cells surviving after insemination, and might increase the number of and the time period during which fertile sperm cells are present in the oviducts.

Effects of *suppressing* uterine contractility have hardly been studied in sows. In luteal sows, which show no uterine activity, inseminated sperm cells can reach the oviducts (First et al., 1968). Suppression of uterine activity by infusion of a β-adrenergic agent before insemination reduced the number of sperm cells at the site of fertilisation, and reduced the relative number of sperm cells entering the oviduct from the uterine horns (Chapter 6). Likely, reduction of uterine contractility prolongs the transit phase in the uterus, reducing sperm quality, and thus reducing the ability of sperm cells to enter the oviducts and fertilise. This is particularly important when insemination takes place long (> 24 hours) before ovulation. On the other hand, 16 to 18 h after insemination sperm cells still capable of in vitro fertilisation can be retrieved from the uterine horns (W. Flowers, personal communication). Whether such sperm cells can still enter the oviducts and fertilise in vivo is not clear from literature. From the work of First et al. (1968) it can be concluded that by 24 h the sperm cells in the uterine horns are no longer motile, and probably hardly any sperm cells enter the oviducts after this time. Within the first few hours after insemination the population of sperm cells in the oviducts increases (Hunter, 1981), and at 2, 8, 14, and 24 h after insemination increasing number of sperm cells are found in the oviducts (Pursel, 1978; Du Mesnil du Buisson & Dauzier, 1955). However, the number of sperm cells in the utero-tubal junction decreases in the same period, contributing to the sperm numbers in the oviduct. It is not clear from which part of the genital tract sperm cells in the oviducts originate over time. A factor that complicates the interpretation of literature data is the

time of recovery relative to ovulation. Around ovulation, the oviducts become more accessible to sperm cells and the migration into the oviducts increases around this time (Mburu et al., 1996). The dynamics of sperm cell distribution therefore depend on the timing of insemination relative to ovulation, but this timing is often not assessed in the available literature.

Stimulation of uterine contractility in sows has been studied far more extensively than suppression. Waberski et al. (1996) observed an increase in the number of accessory sperm cells in the zona pellucida of 3 to 4 days old embryos, when seminal plasma was infused before insemination. Fertilisation was not affected. Infusion of seminal plasma in one horn promoted the transport of sperm cells into the ipsilateral oviduct, during the period of 1 to 6 h after insemination (Viring & Einarsson, 1980). Intravenous injection with 10 IU oxytocin, shortly after insemination with a low volume and semen dose, improved the percentage of fertilised ova from 58% to 72% (Stratman et al., 1959). Probably, stimulation of uterine contractility can improve sperm transport and/or fertilisation. This is important particularly in unfavorable situations, i.e. low number of sperm cells, low volume of inseminate, long interval from insemination to ovulation, etc. However, stimulation of uterine contractility can also impede sperm transport to the oviducts and fertilisation, probably depending on the extent of stimulation. Intra-uterine infusion with 1 mg of cloprostenol before insemination, a dose sufficient to double the frequency of uterine contractions (Chapter 4), increased reflux of semen during insemination, reduced the number of sperm cells in the oviducts, and reduced fertilisation (Chapter 6). Addition of 10 IE oxytocin to the semen dose reduced the percentage of fertilised ova, whereas i.v. injection with the same dose 5 min after insemination improved fertilisation rate (Stratman et al., 1959). The magnitude of uterine contractility after stimulation and probably also the timing of stimulation affect the reflux of semen. This phenomenon might explain the inconsistency founf in the effect of hormonal stimulation of uterine contractility on reproductive performance in field trials (Levis, 2000). Introduction of a boar probably stimulates uterine contractility to an appropriate level, as this increases uterine activity only in sows with low spontaneous uterine activity (Chapter 5). Summarising, a certain degree of uterine contractility is probably important for fast transport of sperm cells to the relatively safe oviducts, especially in situations unfavorable to the chance of

fertilisation. Stimulation of contractility can be favorable to transport of sperm cells and fertilisation to a certain extent. A high level of uterine activity probably reduces uptake of semen by the uterine horns.

An important issue in the concept of sperm transport is the direction in which uterine contractions are propagated along the uterine horns. Uterine contraction waves are thought to originate predominantly from the tubal and cervical ends of the uterine horns, these being the sites with the most pacemaker activity, at least during partus (Taverne, 1982). Tubo-cervical directed contractions are thought to be important to expel seminal plasma after mating (Woelders & Matthijs, 2001), but are probably also important in the distribution of semen over the two horns. With unilateral insemination, fertilisation in the contralateral horn is similar to fertilisation results in the ipsilateral horn, even with low sperm numbers (5×10^7) and a small volume (10 ml) (Martinez et al., 2001). Recently however, evidence has also been given for transport of sperm through the abdominal cavity to the contralateral oviduct (Martinez, personal communications). Cervico-tubal contractions are thought to be important in the transport of semen to the sperm reservoirs. Propagation of contractions depends on communication between myometrial cells, which is increased during estrus, when number of gap junctions are increased due to the high level of estrogens. Observations on the direction of uterine contractions probably depend on the site of measurement. In the tubal end of the horn, for example, cervical-directed contractions probably dominate, because this site is located nearest to the tubal pacemaker zone. Contractions originating cervically are less likely to be propagated all the way to this site than contractions originating tubally. Studies on direction of uterine contractions report tubo-cervical, cervico-tubal, and undirected contractions. Another factor complicating studies on the direction of uterine contractions is the interpretation of tocographs. In the case of several contractions following closely in time, it is difficult to identify in what direction contractions pass the recording sensors, especially when the pattern in luminal pressure or electrical activity is the same for the different contractions. Nevertheless reports on direction of contractility claim that the direction of contractions is a coordinated process, which depends on the stage of estrus (Zerobin & Sporri, 1972; Scheerboom et al., 1987; Brussow et al., 1988) and which can be affected by stimuli involved in mating (Zerobin & Sporri, 1972). Besides regional

differences in pacemaker activity, coordination of the direction of contractions probably depends on the levels of hormones and the location of their receptors on the myometrium. Regional differentiation in the concentration of receptors for various hormones could be responsible for regional differentiation in the sensitivity to those hormones. A higher concentration of oxytocin receptors in the cervical end of the uterus, for example, would implicate a shift to more cervico-tubal directed contractions after oxytocin release in the presence of a boar. The change in direction observed during the various stages of estrus could be related to the change in estrogens, LH etc., changing the origin of contractions according to the localisation of the receptors for these hormones. Unfortunately, no data exist on the regional differences in receptor concentrations, and the implications for direction of uterine contractions. Another factor coordinating uterine contractions might be the local effect of certain stimuli. Sensory stimulation of the cervix might cause an increase in cervically originating contractions. The deposition of seminal plasma in the cervical end of the horns might have a similar effect, at least shortly after insemination. In summary, there is evidence for tubo-cervical, cervico-tubal, and undirected contractility, which are observed simultaneously throughout estrus. More information is needed to elucidate the coordination of contractility during different stages of estrus and the effects of external stimuli.

Conclusions

Spontaneous uterine activity increases during estrus, and has a function in the transport of sperm cells to the oviducts. This is particularly important when other factors around insemination, like semen dose, time of insemination relative to ovulation, etc., are limiting to the number of sperm cells that survive until the time of fertilisation. Variation between sows in uterine activity is consistent throughout estrus, and might contribute to the variation in fertilisation results between sows. Stimulation of uterine contractility around insemination can improve sperm transport to the oviducts, but can also affect sperm transport and fertilisation negatively. The latter occurs when contractility is stimulated to such an extent that the uptake of semen by the genital tract is obstructed and reflux of semen is increased. Introduction of a boar during insemination is probably an adequate way of stimulating contractility, because

oxytocin release is effectively induced in all sows, and because boar presence selectively stimulates contractility in those sows with low spontaneous uterine activity.

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Practical implications

The implications listed below are meant as a translation of the scientific content of this manuscript and serve to indicate the possible applications in modern swine practice. Note that the implications listed here are based on experimental evidence, and therefore practical application should take other factors that are involved into account.

The use of a boar during estrus detection minimises the risk of not detecting estrous sows. Using less stimuli, i.e. estrus detection by an inseminator only or in combination with boar odor, increases the risk of not detecting estrous sows because of a shorter duration of estrus and because such stimuli do not elicit estrous behavior at all in a number of sows. Using more stimuli during estrus detection (e.g. a Detection-Mating Area) does not necessarily add to the efficiency of detection, due to the fact that sows adapt to a higher level of offered stimuli. The use of a boar is also important to support endocrine events leading to ovulation. Boar contact after weaning, a few times a day, can support final follicular growth and increase the number of sows ovulating within a certain window after weaning. This is particularly important in sows that are not able to maintain follicular growth, e.g. due to metabolic constraints.

Timing of ovulation relative to the onset of estrus varies considerably between sows, regardless of the stimuli used to detect estrus. Therefore, onset of estrus is a poor predictor for ovulation. Distinction of different phases during estrus by using two or more different stimuli to detect estrus (e.g. man with boar and man without boar) does not improve the prediction of estrus, because the interval between the different phases is poorly related to the timing of ovulation. Therefore, the use of a boar for estrus detection and the fact that ovulation takes place at a fairly constant two thirds of the way through the period of estrus, is still the best way to retrospectively estimate the time of ovulation.

Uterine contractility is increased during estrus. However, there is huge variation between sows. This might explain some of the variation between sows in fertilisation rate. Uterine contractility is important for fertilisation; suppression of uterine contractility reduces fertilisation rate. Artificial stimulation of uterine contractility to a

high degree, on the other hand, increases the time needed for insemination, increases the reflux of semen during insemination and reduces fertilisation rate. Procedures for insemination should therefore stimulate uterine activity, but preferably in those sows with low uterine activity.

Confrontation with a boar induces an immediate release of endogenous oxytocin in the sow during estrus. In this study manual stimuli, with or without the use of boar odor, did not induce the release of any oxytocin, even if sows showed a standing response. The exhibition of a standing response does therefore offer no indication of whether uterine contractility is also stimulated by applied stimuli. Both endogenously released oxytocin (in reaction to confrontation with a boar) and exogenous oxytocin (5 IU im) increase uterine activity during estrus, but only in those sows that have a below average uterine activity preceding the treatment. In terms of sperm transport, confrontation with a boar around insemination is therefore probably the most appropriate way of stimulating uterine contractility.

Summary

This thesis describes several aspects of the role of the boar in reproductive processes in sows, other than the contribution of male gametes. *Boar stimuli* is the keyword in this. Boar stimuli are sexual stimuli, which are involved in triggering receptive behaviour in sows. Receptive behaviour in sows, the *standing response,* is characteristic for the period of *estrus.* Ovulation takes place two-thirds of the way through estrus, and is an important event for the timing of artificial insemination. However, the duration of estrus is very variable, and therefore the time of ovulation cannot be predicted using the onset of estrus. In the course of estrus, the responsiveness of a sow to boar stimuli first increases and then decreases again. As a consequence, one can define different phases of estrus during which a sow responds with a standing response to different combinations of boar stimuli. The onset of these phases or the time between these phases might be related to the time of ovulation in such a way that ovulation can be predicted more accurately. The first *main aim* of this thesis was to establish a relation between the change in responsiveness to boar stimuli during estrus and the time of ovulation. Besides their role in eliciting and detection of estrous behaviour, boar stimuli are also thought to be important in the *transport* of semen through the female genital tract. This transport is a passive process, for which *uterine contractions* are believed to be responsible. Earlier studies have shown that uterine activity is increased during estrus, but individual variation between sows has hardly been described. Boar stimuli have a stimulatory effect on uterine contractility. However, except for seminal plasma components, there is little information on the effects of boar stimuli on uterine contractility. The second *main aim* of this thesis was to study individual variation in uterine contractility, and to study effects of boar stimuli on uterine contractility.

Estrus and ovulation

In the first two chapters of this thesis an experiment is described, in which different levels of boar stimuli were applied to detect estrus in weaned, multiparous sows. Detection of estrus was performed at 8 h-intervals, and each time all the four different levels of stimuli were applied consecutively for all sows, in order of assumed potency

to induce a standing response. Thus, the change in responsiveness of the sows to the stimuli throughout estrus could be recorded. The four stimulus levels were: 1) a back pressure test (BPT), i.e. manual stimulation of the back and flanks of a sow, 2) confrontation with a teaser boar, in front of a sow's pen, 3) a BPT in the presence of that teaser boar, and 4) a BPT applied after 5 min in a 'detection-mating area' (DMA), a large pen surrounded by four boar pens. The period during which a sow showed a standing response varied with the level of boar stimuli used for detection: detected duration of estrus was 22 h, 29 h, 42 h, and 55 h for the four stimuli, respectively. The difference in potency of the stimuli to induce estrous behaviour is also apparent from the overall percentage of sows in which estrus was detected: 41 %, 54 %, 89 %, and 97 %. Surprisingly, in a different group of sows that did not experience the DMA as one of the detection stimuli, the percentage of sows detected in estrus using a BPT (68 % with stimulus level 1), and the duration of estrus detected by BPT in the presence of the teaser boar (52 h with stimulus level 3), were higher than in the sows described earlier. Apparently, subjecting sows to extra boar stimuli by using the DMA reduced their responsiveness to lower levels of stimuli. Comparing the two groups, it appeared that the use of a DMA compared to a BPT in the presence of a boar had no additional value in terms of the chance to detect estrus. Concluding, increasing boar stimuli reduces the responsiveness to stimuli used for detection of estrus. A BPT in the presence of a teaser boar is sufficient for detection of estrus.

On average, ovulation occurred 24 h, 23 h, 34 h, and 41 h after the onset of the estrus phases detected with the four consecutive stimuli mentioned above. There was too much variation between sows in the timing of ovulation to use onset of these phases as a predictor for the time of ovulation; standard deviation was 13 h, 15 h, 13 h, and 12 h for the four stimulus levels. The intervals between the different phases of estrus were related to the duration of estrus. For example, the interval between the onset of estrus as detected using a BPT in the DMA and the onset of estrus as detected using a BPT in the presence of a boar was 8 h on average, and was related to the time of ovulation relative to the onset of estrus as detected in the DMA ($b = -0.7$; $R^2 = 0.4$). However, the time between the different intervals did not explain enough variation in the time of ovulation in order to be able to predict ovulation. Concluding, unless there is little variation in the duration of estrus in a sow herd, the onset of estrus cannot be

used as a predictor for ovulation, and recording the change in responsiveness to boar stimuli does not explain enough variation to improve the prediction of ovulation.

On the basis of the first experiment, it was hypothesised that if increasing boar stimuli reduce the responsiveness to boar stimuli, then even a low level of boar stimuli is sufficient for detection of estrus, as long as sows are not confronted with higher stimulus levels. In that case, detection of estrus with only a BPT in sows that experience no boar contact at all, would be be as efficient as detection of estrus in the presence of a teaser boar. In Chapter 3 an experiment is described in which a group of primiparous sows that received no boar contact during 9 d after weaning was compared with a group of sows that were confronted with a boar regularly (three times a day). A BPT in absence of the boar was performed in both groups, a BPT in the presence of a boar only in the latter group. The percentage of sows detected in estrus and the detected duration of estrus using the BPT in absence of a boar was equal for both groups. The duration of estrus as recorded using a BPT in the presence of the boar was longer (56 h vs 38 h). Additionally, the number of sows that came into estrus and ovulated within the 9 d of the experiment was increased in the group that received boar contact (51 % vs 30 %). Initial follicular development in the first four days after weaning was observed in all sows, but boar contact increased the number of sows that maintained follicular growth through to ovulation. Concluding, boar contact during estrus detection is necessary to increase the chance of detecting sows that are in estrus, and additionally, boar contact after weaning stimulates follicular growth in a number of sows that are otherwise incapable of sustaining follicular growth to ovulation.

Uterine contractility

In chapter 4, uterine intraluminal pressure was recorded as indicator of myometrial activity. The technique was based on a device used for non-surgical embryo transfer, and enables an open-end catheter to be passed through the cervix, into the uterine lumen. Using this technique, spontaneous myometrial activity was recorded in multiparous, cyclic sows from 4 d before estrus until 3 d after estrus. During the days before estrus, a number of sows did not show any myometrial activity at all.

Approaching estrus, the number of sows with myometrial activity increased, and during estrus, all sows showed myometrial activity. The frequency and amplitude of myometrial contractions also increased approaching estrus, and were maximal on the first two days of estrus. Variation between sows was large on all days recorded, but the difference between sows was consistent; sows with an initially high level of myometrial activity during the days before estrus maintained the high level. After estrus, myometrial activity declined again. In sows with a duration of estrus of 2 days, myometrial activity started to decline earlier after the onset of estrus than sows with a duration of estrus of 3 days.

To test the hypothesis that *estrogens in boar seminal plasma increase myometrial* activity, a physiological dose of estrogens was infused intrauterine. This increased the frequency of contractions during one hour of recording after infusion. A volume effect was excluded by the absence of an effect of a saline infusion with the same volume. To develop tools for manipulating myometrial activity in further studies, both cloprostenol (a synthetic analogue of pgf2α) and clenbuterol (a β-adrenergic agonist) were tested for their effects on myometrial activity. Cloprostenol increased the frequency and the amplitude of contractions. Clenbuterol reduced the frequency and the amplitude of contractions.

Boar presence is also believed to affect myometrial activity. The presence of a boar induces oxytocin release in the sow, which is a known agonist of myometrial activity. In Chapter 5, several exteroceptive boar stimuli are compared in their effect on oxytocin release, on myometrial activity and on receptive behaviour. During estrus, myometrial activity was recorded and the effect of the following stimuli was tested: 1) a BPT, 2) a BPT after spraying boar odour (5α-androstenon) for 2 sec, and 3) a BPT in the presence of a boar. Oxytocin release was only observed in the presence of a boar. Myometrial activity was stimulated by the presence of the boar, but only in the sows with below average frequency of contractions before the treatment. This phenomenon was also observed in an experiment in which 5 IU oxytocin was injected im; myometrial activity increased only in sows with below average activity. The BPT alone or in combination with boar odour spray had a significant but much smaller effect in such sows. Apparently, the presence of a boar is a far more potent stimulus than a

BPT alone or in combination with odour spray, both in the release of oxytocin and in its effect on myometrial activity. The change in myometrial activity caused by the presence of a boar was not related to the magnitude of oxytocin release. Although BPT did not induce oxytocin release, these stimuli did elicit a standing response in one-third of the sows. Apparently, a standing response can occur without oxytocin being released and without affecting myometrial activity. On the other hand, this experiment gave some indications for a facilitatory role of oxytocin in the induction of a standing response, because the magnitude of oxytocin release in the presence of the boar was related to the time during which the standing response was exhibited (1 to 6 min). Concluding, in terms of stimulation of myometrial activity around insemination, the presence of a 'whole' boar appeared to be far more effective than separate boar stimuli. Uterine contractility is only stimulated by the boar in sows with below average uterine activity.

Finally, the role of myometrial contractions in sperm transport and fertilisation was studied (chapter 6). Multiparous, weaned sows, were treated with GnRH to synchronise ovulation and inseminated at a fixed time relative to ovulation. Ten minutes before insemination, sows were treated with 1) cloprostenol, to stimulate myometrial contractility, 2) saline, or 3) clenbuterol, to suppress contractility. The sows were either slaughtered 8 h before expected ovulation to examine the distribution of sperm cells in the genital tract, or 5 d after ovulation to assess fertilisation rate. Cloprostenol increased the number of sows with vaginal reflux of semen, and increased the time needed to empty the semen container, but reduced the number of sperm cells that reached the oviducts and the percentage of fertilised oocytes (median 21% vs 81% for saline). Clenbuterol reduced the time needed to empty the semen container. The number of sperm cells in the different parts of the genital tract for clenbuterol was not significantly different from the saline treatment. However, relative to the number of sperm cells recovered from the proximal part of the uterine horns, the number of sperm cells in the oviducts were reduced by clenbuterol. Clenbuterol also reduced the percentage of fertilised oocytes (median 26% vs 81% for saline). Apparently, in the clenbuterol treatment the sperm cells were less able to enter the oviducts, although their numbers in the uterine horn were equal to the saline treatment. From these data it was concluded that uterine contractions are necessary for fast transport of sperm

cells to the oviducts. Suppression of uterine contractility by clenbuterol did facilitate the emptying of the semen container, but probably the transport time through the uterine horns was increased. Thus, the sperm cells were exposed to the relatively hostile environment of the uterus for a longer period of time, reducing their capability to enter the oviducts and fertilise. Stimulation of uterine contractility to the level as for the cloprostenol treatment probably frustrates the emptying of the semen container because of increased obstruction of the uterine lumen by contractions. As a result, the time needed to empty the semen container is increased and reflux of semen is increased. Probably, a reduced number of sperm cells are retained by the uterine horns and the number of sperm cells reaching the oviduct and the site of fertilisation is reduced. Concluding, not a maximal but optimal level of uterine contractility seems necessary for uptake and transport of sperm cells to the oviducts. Selective stimulation of sows with below average uterine activity, as observed in the presence of a boar (Chapter 5), might be an optimal way of stimulating contractility around insemination.

In the general discussion, the findings in this thesis are discussed in perspective of the available information in literature. It is concluded that both for induction of estrous behavior, and for the process of sperm transport and fertilisation, boar presence is highly recommendable.

Samenvatting

Deze samenvatting is geen letterlijke vertaling van de Engelse versie, maar bedoeld als samenvatting voor leken op dit onderzoeksgebied.

Een beer is meer dan alleen een leverancier van spermacellen. Dit proefschrift gaat in op de rol van de beer in voortplantingsprocessen bij zeugen tijdens de bronst. Het sleutelwoord hierbij is *beerprikkels*. Beerprikkels zijn sexuele prikkels (geur, aanraken etc.) die een rol spelen bij het induceren van receptief gedrag, de zogenaamde *sta-reflex*. De sta-reflex is een teken dat een zeug bereid is om gedekt te worden. Naast hun rol in het opwekken van receptief gedrag hebben beerprikkels ook invloed op de contractiliteit van de baarmoeder. Dit is waarschijnlijk van belang voor het transport van spermacellen naar de eileiders, en dus voor de bevruchting. Met dit proefschrift wordt beoogd het inzicht in de rol van beerprikkels in het opwekken van receptief gedrag en het transport van sperma in de baarmoeder te vergroten, en daarmee het belang van de beer in de voortplanting te beschrijven.

Beerprikkels en receptief gedrag

De periode waarin een sta-reflex wordt vertoond in aanwezigheid van een beer is de *bronst*, en deze duurt bij het varken gemiddeld zo'n twee dagen. Op twee-derde van de bronst vindt de *ovulatie (= eisprong)* plaats, hetgeen een belangrijk moment is omdat de kans op bevruchting het grootst is als een inseminatie binnen 24 uur vóór ovulatie plaatsvindt. Omdat de bronstduur erg varieert, is het ovulatiemoment niet te voorspellen aan het begin van de bronst. Tijdens de bronst neemt de gevoeligheid van een zeug voor beerprikkels eerst toe en daarna af. Afhankelijk van de sterkte van de beerprikkel en het stadium van de bronst zal een zeug dus al dan niet een sta-reflex vertonen als reactie op die prikkel. Zo kan men de bronst bijvoorbeeld indelen in een fase waarin een zeug 'staat' als ze geconfronteerd wordt met een beer en tegelijkertijd handmatig de druk van een dekkende beer op de rug van de zeug wordt geïmiteerd, en daarbinnen een kortere fase waarin de zeug al staat als alleen die druk op de rug wordt geïmiteerd (tactiele prikkels). Op deze manier deelden Willemse & Boender

(1967[1]) de bronst in in een 'beerfase' en een 'inseminatorfase', waarbij de laatste ongeveer tweederde deel besloeg van de eerste. Door verschillende prikkels te gebruiken kan dus de bronst in verschillende fasen worden ingedeeld. Aan de hand daarvan kan mogelijk het ovulatiemoment beter ingeschat worden, mede omdat de tijd tussen de verschillende fasen een indicatie kan zijn voor de duur van de bronst. Het vinden van een goede voorspeller van het ovulatiemoment was het eerste belangrijke *doel* van dit proefschrift.

In de eerste twee hoofdstukken wordt een experiment beschreven waarin vier verschillende beerprikkels werden gebruikt tijdens de bronstcontrole. Bij iedere bronstcontrole (iedere 8 uur) werden de zeugen achtereenvolgens aan alle vier de beerprikkels onderworpen, in (veronderstelde) volgorde van oplopende sterkte van de prikkel: 1) tactiele prikkels: handmatige stimulatie van de flanken en rug van een zeug, 2) aanwezigheid van een beer voor de box van de zeug, 3) tactiele prikkels in aanwezigheid van die beer, en 4) tactiele prikkels nadat de zeugen 5 min in een 'dekarena' hadden doorgebracht, een ruimte omringd door vier hokken met daarin een beer. De duur van de fase waarin de zeugen met een sta-reflex reageerden, was afhankelijk van de prikkel en was 22, 29, 42 en 55 uur voor prikkel 1) tot en met 4). Het kwam voor dat zeugen geen een keer tijdens de bronst reageerden op een bepaalde prikkel: het percentage zeugen dat wel 'gedetecteerd' werd was 41%, 54%, 89% en 97% voor prikkel 1 t/m 4. Opvallend genoeg was bij een andere groep zeugen in hetzelfde experiment, die alleen werd blootgesteld aan prikkel 1 t/m 3, dit detectiepercentage voor tactiele prikkels (prikkel 1) hoger (68% i.p.v. 41%) en de duur van de fase waarin de zeugen reageerden op prikkel 3 langer (52 uur i.p.v. 42 uur). Bij deze zeugen was de reactie op prikkel 3 even sterk als bij de eerdergenoemde zeugen op de sterkere prikkel 4. Kennelijk verlaagde de blootstelling aan meer beerprikkels (dekarena) in de eerste groep zeugen de gevoeligheid van de zeugen voor beerprikkels van een lager niveau, en voegde de dekarena niets toe aan de duur van de fase waarin de zeugen 'stonden'. In termen van bronstdetectie lijkt het dus voldoende om deze uit te voeren door middel van tactiele prikkels in aanwezigheid van een beer.

[1] Willemse AH & Boender J. 1967. The relation between the time of insemination and fertility in gilts. Tijdschrift diergeneeskunde 92: 18-34.

Het ovulatiemoment lag 24 uur, 23 uur, 34 uur, en 41 uur na het begin van de vier fases. Hiermee kon echter niet het ovulatiemoment voorspeld worden, omdat de variatie in het ovulatiemoment voor alle vier prikkels te groot was; de standaarddeviatie van het ovulatiemoment was 13 uur, 15 uur, 13 uur en 12 uur voor de vier prikkels. Het maakt dus niet uit met welke prikkels de aanvang van de bronst gedefinieerd wordt, dit moment varieert altijd aanzienlijk ten opzichte van het ovulatiemoment. De periode tussen het begin van de ene fase en het begin van een andere fase was wel een indicatie voor het ovulatiemoment, maar dit verband was niet sterk genoeg om het ovulatiemoment nauwkeurig genoeg te kunnen voorspellen. Het indelen van de bronst in verschillende fasen door het gebruik van twee of meer beerprikkels levert niet genoeg informatie op om het ovulatiemoment nauwkeurig genoeg te kunnen voorspellen.

Op basis van het eerder beschreven effect van de dekarena op de gevoeligheid voor beerprikkels werd de hypothese geformuleerd dat als zeugen niet met sterkere beerprikkels in aanraking komen, het gebruik van een lage hoeveelheid prikkels net zo efficient is in het opwekken van bronstgedrag als bij zeugen waarbij sterkere prikkels worden toegepast. In dat geval zou de bronstcontrole uitgevoerd met alleen tactiele prikkels net zo efficient zijn als wanneer dit in combinatie met een beer zou gebeuren. In hoofdstuk 3 wordt een experiment beschreven waarin bij gespeende eersteworpszeugen drie keer per dag bronstcontrole werd gedaan, al dan niet met een beer. In de eerste groep werden alleen tactiele prikkels gebruikt, in de tweede groep werd het bronstgedrag ook gemeten met tactiele prikkels in aanwezigheid van een beer. Met tactiele prikkels werd een bronstduur gemeten van 38 en 39 uur in de twee groepen, en ook het 'detectiepercentage' was vergelijkbaar. In aanwezigheid van de beer werd in de tweede groep echter een bronstduur gemeten van 56 uur. In dit geval had dus een sterkere prikkel wel een toegevoegde waarde, en veroorzaakte het contact met een beer in de tweede groep geen lagere gevoeligheid voor tactiele prikkels. Daarnaast zorgde het contact met de beer in de tweede groep ervoor dat niet 30 %, maar 51 % van de zeugen berig werden binnen 9 dagen na spenen. Bij alle zeugen werd met behulp van echografie in de eerste dagen na spenen follikelgroei op de eierstokken vastgesteld. Bij een gedeelte van de zeugen stagneerde deze groei echter na een paar dagen en leidde niet tot ovulatie. Het contact met de beer zorgde

er kennelijk voor dat in een aantal zeugen de follikelgroei gestimuleerd werd en resulteerde in ovulatie. Het gebruik van een beer lijkt dus aan te bevelen voor bronstcontrole en voor het stimuleren van follikelgroei.

Baarmoedercontractiliteit

Spermacellen die bij een natuurlijke dekking of kunstmatige inseminatie in het begin van de baarmoeder geloosd worden, moeten vervolgens door de baarmoederhoornen (ca 1 m lang) naar de eileiders getransporteerd worden. Verondersteld wordt dat baarmoedercontracties hierin een belangrijke rol spelen. In hoofstuk 4 wordt een niet-chirurgisch techniek beschreven waarmee de druk in de baarmoeder gemeten kan worden. Hiertoe wordt een dunne, met vloeistof gevulde catheter door de baarmoederhals in de baarmoeder gebracht. Met deze techniek is de spontane baarmoedercontractiliteit rond de bronst gemeten (hoofdstuk 4). De baarmoeder is namelijk in staat autonoom te functioneren, hetgeen kenmerkend is voor gladde spieren zoals de baarmoederspieren. In de dagen voor de bronst bleek een deel van de zeugen totaal geen baarmoedercontractiliteit te vertonen. Naarmate de bronst naderde nam dit gedeelte af en tijdens de bronst vertoonden alle zeugen baarmoederactiveit. De frequentie (aantal per uur) van contracties en de sterkte van de contracties vertoonde een zelfde patroon: deze namen toe naarmate de bronst naderde en waren maximaal tijdens de bronst. Na de bronst nam de baarmoedercontractiliteit weer af. Opvallend was de grote variatie tussen zeugen in baarmoedercontractiliteit. Het verschil tussen de zeugen was wel consistent rondom de bronst: zeugen met een relatief lage baarmoedercontractiliteit in de dagen voor de bronst hielden een relatief lage baarmoedercontractiliteit in de daaropvolgende dagen. Bij zeugen met een langere bronstduur (3 dagen i.p.v. 2 dagen) was de periode van verhoogde baarmoedercontractiliteit ook langer.

Beerprikkels kunnen de baarmoederactivititeit beinvloeden en kunnen zo mogelijk spermatransport door de baarmoederhoorn en de bevruchting beinvloeden. Spermaplasma bevat bijvoorbeeld stoffen die de baarmoeder stimuleren en de contractiliteit verhogen. Over het effect van uitwendige prikkels op de baarmoedercontractiliteit, zoals de geur van een beer of tactiele prikkels, is echter

weinig bekend. In dit proefschrift wordt van een aantal beerprikkels de invloed op de afgifte van oxytocine, op de baarmoedercontractiliteit en op het bronstgedrag beschreven (hoofdstuk 5). Oxytocine is een stof die baarmoedercontractiliteit stimuleert, en die wordt afgegeven bijvoorbeeld als reactie op de geur van een beer. In dit proefschrift werden de volgende prikkels vergeleken: 1) tactiele prikkels, 2) tactiele prikkels, nadat een component van beregeur (5α-androstenon) vóór de zeug was verneveld, of 3) tactiele prikkels in aanwezigheid van een beer. De aanwezigheid van een beer veroorzaakte bij alle zeugen een sta-reflex en een duidelijke afgifte van oxytocine. De aanwezigheid van een beer stimuleerde ook duidelijk de baarmoedercontractiliteit, maar alleen bij zeugen die voor het blootstellen aan de prikkel een lager dan gemiddelde baarmoedercontractiliteit hadden. Dit laatste fenomeen werd ook waargenomen in een ander experiment, waarbij oxytocine werd ingespoten; ook hier werd alleen een effect waargenomen bij de zeugen met een beneden gemiddelde baarmoedercontractiliteit. Tactiele prikkels, alleen of in combinatie met 5α-androstenon, hadden geen afgifte van oxytocine tot gevolg en hadden slechts een gering effect op de baarmoedercontractiliteit. Wel liet een derde van de zeugen een sta-reflex zien als reactie op deze prikkels. Het vertonen van een sta-reflex hoeft dus niet gepaard te gaan met de afgifte van oxytocine en een verhoogde baarmoedercontractiliteit. Aan de andere kant leek oxytocine een faciliterende rol te hebben in het vertonen van de sta-reflex: hoe hoger de afgifte van oxytocine in aanwezigheid van de beer, hoe langer de positie van de sta-reflex 'vastgehouden' werd. In termen van oxytocineafgifte, stimuleren van baarmoedercontractiliteit, en het opwekken van bronstgedrag is de aanwezigheid van een beer dus een duidelijk sterkere prikkel dan tactiele prikkels en 5α-androstenon.

De rol van baarmoedercontracties in het transport van sperma en bevruchting is in het kader van dit proefschrift onderzocht door rond inseminatie de baarmoedercontractiliteit te onderdrukken dan wel te stimuleren. Dit werd bereikt door tien minuten vóór de eigenlijke inseminatie zeugen in de baarmoeder te infuseren met een stof die de baarmoedercontractiliteit stimuleert dan wel onderdrukt, of met een placebo. Stimulatie van de baarmoedercontractiliteit verlengde de tijd die nodig was om de spermadosis in de zeug te laten 'lopen', en verhoogde de terugvloei van sperma. Dit resulteerde in een lager aantal spermacellen dat de eleiders bereikte,

evenals een lager percentage bevruchte eicellen. Kennelijk bemoeilijkte het frequent contraheren van de baarmoeder de opname van het sperma. Onderdrukking van de baarmoedercontractiliteit zorgde juist voor een snellere opname van de spermadosis, maar het aantal spermacellen dat het laatste deel van de baarmoederhoorn en de eileider bereikte was ongeveer gelijk aan dat bij de placebo behandeling. Van het aantal spermacellen dat het laatste gedeelte van de baarmoederhoorn bereikte kwam er echter een *relatief* lager aantal in de eileiders terecht. Kennelijk waren deze spermacellen minder in staat om de eileiders te bevolken. Het percentage bevruchte eicellen was ook lager dan bij de placebo groep. Gedacht wordt dat onderdrukking van de baarmoedercontractiliteit het transport richting de eileiders vertraagde, waardoor de spermacellen langer blootstonden aan aan het minder gunstige baarmoedermilieu, en zodoende van minder kwaliteit waren. Baarmoedercontracties blijken dus wel degelijk nodig voor spermatransport en bevruchting. Het is echter niet 'hoe meer baarmoedercontracties hoe beter': boven een bepaald niveau aan baarmoedercontractiliteit neemt de terugvloei van sperma toe en wordt de opname en het transport van sperma verstoord.

Conclusie

De aanwezigheid van een beer is voor het opwekken van receptief gedrag, maar ook voor het stimuleren van de baarmoedercontractiliteit een noodzakelijke maar ook voldoende prikkel. Baarmoedercontractiliteit is nodig voor spermatransport en bevruchting, maar een te hoge mate van baarmoedercontractiliteit werkt averechts. Selectieve stimulatie van zeugen met een lager dan gemiddelde baarmoedercontractiliteit, zoals in aanwezigheid van een beer het geval is, zou wel eens optimaal kunnen zijn in termen van spermatransport en bevruchting. In dat geval lijkt het dan ook aan te bevelen om niet alleen bij de bronstcontrole maar ook bij de inseminatie stimulatie met een beer te gebruiken.

Curriculum Vitae

Pieter Langendijk werd op 7 november 1971 geboren in Ndanda, Tanzania. In 1990 behaalde hij het VWO diploma aan het Florens Radewijns College in Raalte. Na het diploma 'Nederlandse Landbouw' behaald te hebben aan de Internationale Agrarische Hogeschool Larenstein (Deventer) vervolgde hij zijn studie in Wageningen en studeerde begin 1997 af als Zoötechnicus. In verband met de uitbraak van varkenspest in Nederland vervulde hij enige maanden een functie bij mengvoedercoöperatie Cehave in Veghel. In september 1997 begon hij bij de toenmalige Vakgroep Veehouderij van de Universiteit Wageningen aan het promotie-onderzoek waarvan dit proefschrift het resultaat is. Vanaf 15 october 2001 is Pieter Langendijk werkzaam aan de Faculteit Diergeneeskunde in Utrecht.

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