INFLUENCE OF INSEMINATION CONDITIONS ON EARLY PREGNANCY IN PIGS, WITH EMPHASIS ON EMBRYONIC DIVERSITY

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INFLUENCE OF INSEMINATION CONDITIONS ON EARLY PREGNANCY IN PIGS, WITH EMPHASIS ON EMBRYONIC DIVERSITY

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Proefschrift

ter verkrijging van de graad van
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op gezag van de rector magnificus,
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op vrijdag 11 september 1992
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

VOORWOORD

Resultaat van 4 jaar onderzoek bij de vakgroep Veehouderij van de Landbouwuniversiteit te Wageningen; een proefschrift vol variatie ! Voor degenen die minstens de samenvatting lezen zal duidelijk zijn dat variatie ongewenst kan zijn wanneer het een toom varkensembryo's betreft. Ook zal hen duidelijk zijn dat die binnen-toom variatie beinvloed kan worden door stimulatie van buitenaf. Ten aanzien van dit proefschrift deed de stimulatie zich voor in de vorm van zeer veel personen, zowel op het werk als daarbuiten. Velen hebben op de een of andere wijze bijgedragen aan de totstandkoming van dit proefschrift. Ik wil er een aantal bij name noemen.

Allereerst mijn promotor, Prof. dr. J.P.T.M. Noordhuizen. Jos, we zijn op dezelfde dag, 1 maart 1988, begonnen met ons werk bij de vakgroep Veehouderij. Door de aanwezigheid van de twee co-promotoren, was je niet betrokken bij de dagelijkse begeleiding. Maar als het nodig was, was je er. Bijvoorbeeld op het moment dat het gebruik van de echografie dreigde te mislukken !

Twee co-promotoren, Dr. ir. T. van der Lende en Dr. ir. B. Kemp, wat een luxe !

Tette, jij stond aan de basis van het projectvoorstel dat uitmondde in dit proefschrift. Ik ben bang dat ik verantwoordelijk ben voor je eerste grijze haren. Bijvoorbeeld door het inleveren van concept-artikelen met 'slordigheden'. Tette, bedankt voor de aandachtige en kritische wijze waarop je het onderzoek en de papierstroom 4 jaar lang hebt gevolgd.

Bas, toen Tette naar het IVO-DLO vertrok en jij zijn plaats op de vakgroep innam, kreeg je een paar erfenissen. Dit onderzoek was er één. Ik dank je voor het nooit aflatende enthousiasme waarmee je die erfenis tot je (mede) verantwoordelijkheid hebt gemaakt. Je positieve instelling is zeer stimulerend en ik hoop daar nog een poosje van te profiteren.

De werkgroep 'Vroege Dracht' bestaat niet meer. Niettemin hebben in de loop der jaren meerdere leden een bijdrage geleverd aan dit proefschrift. Henri Stroband, Truus te Kronnie, Marleen Boerjan, Frans Helmond en John Mattheij, bedankt. Ook heb ik gedurende een korte periode een heuse begeleidingsgroep gehad waarin naast reeds genoemde personen, ook André Henken en Willem Schouten zitting hadden. Mede door hun toedoen zijn in die periode Hoofdstuk 2 en 3 in vele concepten verschenen.

Het klinkt afgezaagd, maar zonder studenten en stagiaires had ik het echt niet gered. Ik noem ze in volgorde van aanwezigheid: Coen Nieuwenhuis, Roland Jansen, Hans Spoolder, Margot van Lange, Ronald Siemonsma, Martin Demmer, Miriam de Koning, Evert-Jan Beumer, Ge-Anneke de Vries, Susan van den Hoven, Hendrik de Vor en Gerjan van Alst. Voor de meesten betekende dat dagen èn nachten naar echobeelden van varkensovaria turen, wat een (afstudeer) vak !

STELLINGEN

1. De invloed van de beer op reproductieprocessen van zeugen bestaat uit meer dan de participatie van zijn spermacellen in de bevruchting.
   Dit proefschrift

2. Het ontbreken van een relatie tussen de ovulatieduur en binnen-toom variatie in embryonale ontwikkeling suggereert dat de ovulatieduur niet is gerelateerd aan embryonale sterfte welke een gevolg is van deze variatie.
   Dit proefschrift

3. De aanname van Pope et al. (1990) dat de fertilisatieduur geen invloed heeft op binnen-toom variatie in embryonale ontwikkeling is op zijn minst voorbarig en behoeft nader onderzoek.
   Pope, W.F., Xie, S., Broermann, D.M., Nephew, K.P., 1990. J. Reprod. Fertil, Suppl. 40: 251-260.
   Dit proefschrift

4. Transrectale echografie is een goede methode om het ovulatiemoment en de ovulatieduur bij varkens te bepalen en biedt daarmee nieuwe mogelijkheden tot het evalueren van inseminatiestrategieën.
   Dit proefschrift

5. Een succesvolle methode om spermacellen te scheiden op basis van geslachtschromosomen of daaraan gerelateerde kenmerken (het 'sexen van sperma') zal in de varkenshouderij leiden tot een relatieve toename in het gebruik van kunstmatige inseminatie.

6. Het gebruik van niet-chirurgische embryotransplantatie is in de varkenshouderij, in tegenstelling tot in de rundveehouderij, afhankelijk van de *in vitro* productie van embryo's.

7. Resultaten van epidemiologisch onderzoek kunnen een bijdrage leveren aan de hypothese-vorming ten behoeve van fundamenteel fysiologisch onderzoek.

8. Het schrijven van een proefschrift geldt als een proeve van bekwaamheid tot het zelfstandig beoefenen van de wetenschap (Promotiereglement Landbouwuniversiteit, vastgesteld op 20 februari 1990).
Niettemin is goede begeleiding hierbij cruciaal.

9. Wetenschap en Geloof lijken in die zin op elkaar dat beide meer vragen oproepen dan in eerste instantie beantwoord worden.

10. Iemand 'de waarheid zeggen' kan ook tactvol.

11. Assertiviteit is een vorm van zelfverdediging.

12. Een kamer met vrouwen en een P.C. is niet per definitie het secretariaat.

13. Een scheidsrechter moet zich niet uit het veld laten slaan.

N.M. Soede
Influence of insemination conditions on early pregnancy in pigs,
with emphasis on embryonic diversity
Wageningen, 11 september 1992.

Alle experimenten zijn uitgevoerd op proefaccomodatie 'de Haar'. Velen hebben daarbij een handje geholpen, maar met name Jan Hagens, Ries Verkerk, André Jansen en Ben van de Top hebben veel gedaan. Dank jullie wel.

In het tweede deel van het onderzoek (Hoofdstuk 6 en 7) is het gebruik van echografie onontbeerlijk geweest. Ik dank de bond van verenigingen voor K.I. van Varkens voor het beschikbaar stellen van een echograaf voor dit onderzoek. We houden hem nog even !

Nanette van Hapert heeft de 'laatste' aanpassingen aan de layout verzorgd. Nanette, bedankt voor je tijd en je geduld.

Op 11 september heb ik twee paranimfen die mij bij kunnen staan, Wouter Hazeleger en Ger de Vries Reilingh. Wouter, daarmee ben je van het prille begin tot het (bittere ?) einde nauw bij het onderzoek betrokken geweest. Betrokkenheid bij ondermeer de experimenten en bij het doorlezen van concepten. Ook heb ik je vaak als praatpaal gebruikt. Dankjewel voor al die functies. Ger wil ik bedanken voor de gezelligheid en de vriendschap van de laatste jaren, zowel op het werk als daarbuiten.

Tot slot wil ik mijn ouders bedanken. Zij huldigen het standpunt dat je 'nooit te veel kunt leren'. Verder zal het feit dat we thuis 'varkens hadden' ongetwijfeld hebben bijgedragen aan de keuze voor de veeteeltstudie en meer specifiek het varkensonderzoek.

Nicoline Soede

'The great tragedy of science:
the slaying of a beautiful hypothesis by an ugly fact'

(T.H. Huxley, Collected essays)

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Soede, N.M., 1992. Influence of insemination conditions on early pregnancy in pigs, with emphasis on embryonic diversity (Invloed van inseminatiecondities op de vroege dracht bij het varken, met nadruk op variatie in embryonale ontwikkeling).
In pig husbandry, reproductive performance (litter size, pregnancy rate) after either artificial insemination or natural mating is considered to be similar. However, under experimental conditions, boar stimulation around insemination has been found to influence reproductive performance. For the present study, it was hypothesized that boar stimuli around insemination affect reproductive parameters during early pregnancy. Effects on embryonic diversity were emphasized because of its suggested relationship with embryonic mortality. In the first part of the present study it was found that insemination conditions affect parameters such as the duration of oestrus, accessory sperm count of the embryos and embryonic diversity, presumably through an influence on sperm transport and the process of ovulation. The effects were partly dependent on the social conditions of the gilts. In the second part of the study, attention was given to the process of ovulation. The duration of ovulation was of interest because of its suggested relationship with embryonic diversity. Transrectal ultrasonography was developed to study the onset and duration of ovulation in individual animals. Embryonic diversity did not appear to be related to the duration of ovulation, but was related with the accessory sperm count, suggesting a relation between the duration of fertilization and embryonic mortality.
Ph.D. thesis, Department of Animal Husbandry, Agricultural University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

CONTENTS

GENERAL INTRODUCTION

GENERAL INTRODUCTION

In pig husbandry, reproductive performance after either natural mating (NM) or artificial insemination (AI) is considered to be similar. Experiments concerning influences of boar stimuli at or around insemination show effects of specific stimuli on several reproductive processes (e.g. sperm transport and ovulation process) in oestrous sows and gilts (e.g. Ziecik et al., 1981; Claus et al., 1989; Claus, 1990; Weitze et al., 1990a, b). Consequently, reproductive performance may be affected. Hemsworth et al. (1978), showed that, in pairwise housed sows, litter size was significantly increased when sows were given boar contact prior to AI. In contrast, in individually housed gilts, the boar contact caused a significant decrease in litter size. Therefore, insemination conditions (boar stimulation in combination with social/housing conditions) may affect reproductive performance in pigs.

Variation in litter size as found by Hemsworth et al. (1978) presumably are mainly due to embryonic mortality, i.e. prenatal mortality during the first 30 to 40 days of pregnancy. Embryonic mortality is a more important determinant of litter size in pigs than ovulation rate (Leymaster et al., 1986). Embryonic mortality in pigs accounts for the loss of on average 20% to 40% of the fertilized oocytes (Hughes and Varley, 1980; Pope and First, 1985; Van der Lende and Schoenmaker, 1991) and causes an important part of reproductive losses. Causes of embryonic mortality are poorly understood. It has been made plausible, using embryo transplantation experiments, that within-litter embryonic diversity is of major importance for embryonic losses occurring after day 11 of pregnancy (Pope et al., 1982; Pope et al., 1986). Embryonic diversity, in turn, was found to be causally related to the duration of ovulation (Pope et al., 1988; Xie et al., 1990). Large within-litter embryonic diversity in pigs is reported from the early embryonic phase (Xie et al., 1990), throughout pregnancy (e.g. Perry and Rowlands, 1962; Anderson, 1978; Pusateri et al., 1990) up to the late foetal stage (Van der Lende, 1989). Neither the possibility to influence physiological within-litter embryonic diversity, nor the importance of the duration of ovulation as a cause of within-litter embryonic diversity under physiological conditions has been investigated.

The following hypothesis has been formulated for the present study, based on the above: 'Boar stimuli around insemination and social/housing conditions of sows/gilts influence reproductive parameters during early pregnancy'. Effects on within-litter embryonic diversity are emphasized. Inconsistent results of the experiments investigating this hypothesis, made clear that in studies investigating such influences of boar stimulation, it would be necessary to assess the moment of ovulation (time of ovulation of first oocyte) and the duration of ovulation (interval between the ovulation of the first and last oocyte) in individual sows. No such non-invasive method was available. Therefore, a non-invasive method was developed and subsequently used to study the duration of ovulation as a cause of within-litter embryonic diversity under physiological conditions.

In Chapter 1, a review is given of literature concerning influences of boar stimulation around insemination on reproductive processes, such as standing response, sperm transport, ovulation, fertilization, pregnancy and reproductive performance at farrowing. Furthermore, influences of social/housing conditions of sows are presented and discussed.

In a first experiment, effects of boar stimulation after AI on reproductive parameters during early pregnancy was studied in gilts that had been reared and housed either individually or pairwise. Gilts were slaughtered at Day 9-11 after insemination. Effects on early reproductive performance in terms of pregnancy rate, embryonic mortality rate and average embryonic development are described in Chapter 2, effects of the social/housing conditions on mating behaviour are presented in Chapter 3, and effects on within-litter embryonic diversity in Chapter 4.

Based on the results of the first experiment, a second experiment was performed to verify the effects of boar stimulation after AI. Additionally, effects of boar stimulation after AI were studied during an earlier stage of pregnancy. The second experiment involved individually reared and housed gilts only. The gilts were slaughtered at Day 5/6 or 11 after insemination. The results concerning within-litter embryonic diversity and early reproductive performance are described and discussed in Chapter 5.

Effects of boar stimulation on reproductive parameters were not consistent in these two experiments (Chapter 2 - 5). Therefore, a method was developed to assess the onset of ovulation and the interval between first and last ovulation (the duration of ovulation) in individual sows, in order to measure more direct effects of boar stimulation.

Assessment of the duration of ovulation necessitates the repeated assessment of an accurate follicle count of both ovaries at short time intervals. In Chapter 6, the reliability of transrectal ultrasonography to assess the duration of ovulation is evaluated and first results concerning the duration of ovulation and its relation with early within-litter embryonic diversity at approximately 100 hours after ovulation are presented and discussed.

Chapter 7 describes an experiment in which influences of insemination on the duration of ovulation were investigated using the technique as described in Chapter 6. Gilts were slaughtered at approximately 120 hours after ovulation to study within-litter embryonic diversity. Furthermore, possible effects of transrectal ultrasonography on early reproductive performance were investigated.

In the General Discussion, the major findings of Chapters 2 - 7 will be discussed. A review of literature is incorporated concerning the experiments that have led to the hypothesis that within-litter embryonic diversity leads to embryonic mortality and that this is caused by the duration of ovulation. Subsequently, possible causes of differences in embryonic diversity between litters are outlined and implications of the present experiments concerning the hypothesized relation between the duration of ovulation and embryonic diversity are discussed.

REFERENCES

Claus, R., Moshammer, T., Aumüller, R. and Weiler, U., 1987. Replenishment of AI-doses with oestrogens in physiological amounts: effect on sow prolificacy in a field trial. J. Vet. Med. A., 36: 797-800.

Claus, R., 1990. Physiological role of seminal plasma components in the reproductive tract of the female pig. J. Reprod. Fertil., Suppl. 40: 117-131.

Hemsworth, P.H., Beilharz, R.G. and Brown, W.J., 1978. The importance of the courting behaviour of the boar on the success of natural and artificial matings. Appl. Anim. Ethol., 4: 341-347.

Hughes, P.E. and Varley, M.A., 1980. Factors affecting embryonic and foetal mortality. In: P.E. Hughes and M.A. Varley (Editors). Reproduction in the pig. Butterworths, London, UK, pp. 107-118.

Leymaster, K.A., Christenson, R.K. and Young, L.D., 1986. A biological model to measure uterine potential for litter size in swine. 3rd World congress on genetics applied to livestock production. XI. Genetics of reproduction, lactation, growth, adaptation, disease, and parasite resistance. Lincoln, Nebraska, pp. 209-214.

Pope, W.F., Maurer, R. and Stormshak, F., 1982. Survival of porcine embryos after asynchronous transfer. Proc. Soc. Exp. Biol. Med., 171: 179-183.

Pope, W.F., Lawyer, M.S., Nara, B.S. and First, N.L., 1986. Effect of asynchronous superinduction on embryo survival and range of blastocyst development in swine. Biol. Reprod., 35: 133-137.

Pope, W.F., Wilde, M.H. and Xie, S., 1988. Effect of electrocautery of nonovulated Day 1 follicles on subsequent morphological variation among Day 11 porcine embryos. Biol. Reprod., 39: 882-887.

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

Van der Lende, T. and Schoenmaker, G.J.W., 1990. The relationship between ovulation rate and litter size before and after Day 35 of pregnancy in gilts and sows: an analysis of published data. Livest. Prod. Sci., 26: 217-229.

Weitze, K.F., Rabeler, J., Willmen, T. and Waberski, D., 1990a. Interaction between inseminate, uterine and ovarial function in the sow. I. Influence of seminal plasma and oestrogens in the inseminate on intragenital sperm transport, time of ovulation and fertility results in gilts. Reprod. Dom. Anim., 25: 191-196.

Weitze, K.F., Lotz, J., Everwand, S., Willmen, T. and Waberski, D., 1990b. Interaction between inseminate, uterine and ovarial function in the sow. II. Investigations into the influencing of ovulation by the use of sperm free media. Reprod. Dom. Anim., 25: 197-204.

Xie, S., Broermann, D.M., Nephew, K.P., Geisert, R.D. and Pope, W.F., 1990. Ovulation and early embryogenesis in swine. Biol. Reprod., 43: 236-240.

Ziecik, A., Tilton, J.E. and Williams, G.L., 1981. Effect of mating on the luteinizing hormone surge in the pig. J. Anim. Sci., 53: 434-438.

Chapter 1

BOAR STIMULI AROUND INSEMINATION AFFECT REPRODUCTIVE PROCESSES IN PIGS. A REVIEW

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BOAR STIMULI AROUND INSEMINATION AFFECT REPRODUCTIVE PROCESSES IN PIGS. A REVIEW

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ABSTRACT

Evidence is presented that specific boar stimuli at or around the moment of insemination influence reproductive processes such as sperm transport and the ovulation process and consequently affect reproductive performance. Besides external stimulation (like olfactory and tactile stimuli), also internal stimulation (like sperm plasma and some of its components) is important. Furthermore, it is demonstrated that e.g. social conditions of sows may alter the effects of boar stimulation on reproductive processes.

Keywords: boar stimuli, insemination, ovulation, reproduction.

INTRODUCTION

The influence of boar stimuli on puberty attainment in gilts is well known; daily introduction of a mature boar to prepuberal gilts from an age of approximately 160 days onwards will advance the attainment of puberty (reviewed by Dyck, 1988). Furthermore, specific boar stimuli are known to be of importance for the expression of the standing response in oestrous sows (Signoret, 1970, 1971a, b). Although the exact mechanism(s) of these phenomena are still unclear, several boar stimuli are reported to be of importance (Hughes et al., 1990; Signoret, 1970, respectively).

In addition to the above mentioned effects of boar stimuli, evidence is accumulating that boar stimuli at or around the moment of insemination may influence specific reproductive processes. This review briefly describes boar stimuli and, subsequently, focuses on influences of boar stimuli at or around insemination on sperm transport, the process of ovulation and on subsequent reproductive performance. Furthermore, attention is given to the influence of social conditions of gilts and sows on these processes. Female pigs will be referred to as sows, including both sows and gilts, unless specified differently.

BOAR STIMULI

Boar stimuli are normally divided in olfactory, tactile, visual and auditory stimuli (Willemse and Boender, 1966; Schenk, 1967; Signoret, 1970; Hughes et al., 1990). Stimulus values of olfactory aspects of the boar have been studied intensely (reviewed by Perry, 1982). During courting, the boar emits a frothy saliva produced in the submaxillary salivary glands and may urinate frequently (Signoret, 1970). The saliva and urine of mature boars (older than 9 months) contain large quantities of the pheromones 5α-androstenone and 3α-androstenol. Both pheromones were found to have a signalling function, whereas only the latter seems to have a primer function (reviewed by Booth, 1988 and Hughes et al., 1990). Tactile contacts are initiated frequently by the boar during courting and are mainly directed towards the snout, the ano-genital region and the sides of the sow (Signoret, 1970, 1971a, b). The intensity of these contacts is illustrated by the fact that the boar often partly lifts the sow. During courting, the boar, furthermore, grunts characteristically which may act as an auditory stimulus (Signoret, 1970).

Additional stimulation of the sow takes place during mating. For example, (parts of) the internal reproductive tract are stimulated by tactile contact. This tactile stimulation involves the penis of the boar (vagina and cervix) and the volume of the ejaculate (part of the cervix, uterus, oviduct). Furthermore, specific sperm plasma components may act as chemical stimuli.

Between boars, differences in stimulus value exist. Hughes et al. (1985) demonstrated that the stimulus value of boars depends on age. The proceptivity and sexual receptivity of gilts was increased when olfactory and auditory stimuli were added to 6 to 7 months old boars but not when added to 9 to 10 months old boars. Boars of the same age may differ in libido (see review by Wodzicka-Tomaszewska et al., 1981), thereby possibly differing in stimulus value. Furthermore, indications exist that both boars and sows show partner preference (Tanida et al., 1991). This consequently suggests that psychological mechanisms also determine the stimulus value of a boar for an individual sow.

REPRODUCTIVE CHARACTERISTICS INFLUENCED BY BOAR STIMULI AROUND INSEMINATION

Standing Response

For a successful natural mating, the sow has to show a standing response to allow the boar to mate. Although the exact physiological mechanism of this standing response is still unknown, especially olfactory and tactile stimuli of the boar are involved (reviewed by Signoret, 1970, 1971a; Perry et al., 1980). Signoret and du Mesnil du Buisson (1961 in

Signoret, 1970) reported that 60% of the oestrous sows showed a standing response to the Back Pressure Test (BPT) when applied in absence of a boar. Introduction of both olfactory and auditory stimuli increased this to 90%, which is comparable to the 95% found by Willemse and Boender (1966) under similar conditions. Addition of visual and tactile boar stimuli increased the 90% with an additional 7% and 3%, respectively. The importance of olfactory stimulation was, furthermore, shown by Signoret and Bariteau (1975); approximately 50% of the sows that were negative to the BPT showed a standing response after spraying 16-androstenes in front of the sows' snout without negative effects on fertility.

The period during which the standing response can be evoked by the boar is longer than that during which it can be evoked by BPT, the ratio between the lengths of the periods being approximately 3:2 in a situation where olfactory and auditory boar stimulation are provided during the BPT (Willemse and Boender, 1966). However, a high degree of boar contact over a longer period may have negative effects. For example, housing of sows adjacent to boars (high degree of contact) decreased the duration of oestrus and oestrus detection rate compared to housing of sows opposite to boars (Hemsworth et al., 1988; Hemsworth and Barnett, 1990). The authors suggested that this was caused by habituation to the boar.

It can be concluded, that especially the tactile and olfactory stimulation by the boar are of importance for the standing response.

Sperm Transport

Sperm transport is defined as the movement of sperm by the female reproductive tract from the site of deposition of semen (cervix and corpus uteri) to the site of fertilization (ampulla of the oviduct), therefore not including the sperms' intrinsic motility. The transport of sperm cells to the utero-tubal junction is mainly due to uterine contractions (Dauzier and Du Mesnil du Buisson, 1956 and Mann et al., 1956, both in Blandau and Gaddum-Rosse, 1974; Hunter,1973 in Claus et al., 1989b). Transport of sperm cells through the oviduct depends on ciliary activity of the epithelial cells (Fléchon and Hunter, 1981), oviductal contractions and attendant fluid currents (Blandau and Gaddum-Rosse, 1974; Hunter et al., 1983). Intrinsic sperm motility is essential for the passage of the sperm cell through the zona pellucida (see review by Hawk, 1983).

Many experiments have been performed to study mechanisms of and influences on sperm transport. In these experiments, which will be discussed hereafter, a number of criterions have been used to evaluate sperm transport. The most direct measure for sperm transport is to determine the distribution of sperm cells in the reproductive tract within at a fixed time after mating. Indirect measures of sperm transport are e.g. the number of eggs fertilized (within a certain limited time after insemination), the number of accessory sperm cells in the zona pellucida, the myometrial activity of the uterus and the oviduct, and oxytocin

release. The latter is known to increase both uterine and oviductal contractions (Reeves, 1987).

The effect of a number of boar stimuli on sperm transport have been evaluated. Mattioli et al (1986) sprayed 5α-androstenone (one of the main olfactory boar stimuli) in front of the sows' snout during 2 seconds and found an oxytocin release within 2 minutes. This may subsequently lead to uterine contractions as was found after boar introduction to a sow by Döcke and Worch (1963). The latter authors also found, that when sows were fixated, artificial insemination did not affect uterine motility. Additional clitoris massage resulted in a single contraction, whereas nosing activity of a vasectomized boar resulted in prolonged contraction activity, which was increased by mounting and peaked during mating. This high response during mating may well be associated with the immediate 20-fold rise in peripheral oxytocin levels found by Claus and Schams (1990, in Weiler and Claus, 1991) during mating. When sows were not fixated, even artificial insemination positively affected uterine motility and effects of all boar stimuli were stronger and lasted longer compared to the effects in fixated sows (Döcke and Worch, 1963).

Drugs that are known to stimulate contractions of smooth muscle cells have been added to an insemination dose to study their effects on sperm transport and reproductive performance. Compared to control sows, addition of carbacholine (1 mg) significantly increased the number of sperm cells in the oviduct and the number of accessory sperm cells in the zona pellucida, both at 16 hours after ovulation, while oxytocin (50 I.U.) gave intermediate results (Baker et al., 1968). In a number of experiments, addition of oxytocin to the inseminate had positive effects on reproductive performance, concerning both farrowing rate (Sergeev, 1963; Krajńák, 1988 (only significantly in sows); Odehnal et al., 1989) and litter size (Sergeev, 1963; Krajńák, 1988 (only in sows)).

Of the boar stimuli at mating, the role of seminal plasma in sperm transport has been studied intensively. Rath et al. (1989) inseminated 36 gilts with either seminal plasma or buffer immediately prior to artificial insemination. At Day 3-5 after insemination, the number of accessory sperm cells in the zona pellucida was higher in the gilts that were pre-inseminated with seminal plasma. A component of seminal plasma that may be responsible for this effect is oestrogen (reviewed by Claus et al., 1987; Claus, 1990). Oestrogens are present in large quantities in the seminal plasma of boars, but the total quantity per ejaculate may vary considerably from approximately 0.5 μg to 15.3 μg, depending on season and boar (Claus et al., 1987). Of the total amount of oestrogens, approximately 85% originates from the Leydig cells and approximately 15% from the accessory sex glands (Claus et al., 1985 in Claus, 1990). Claus et al. (1987, 1989b) compared myometrial activity of control animals (no insemination) with inseminated gilts (either saline or saline plus oestrogens) and found that myometrial activity was increased for 3 hours following insemination with saline plus oestrogens, but not following insemination with only saline, suggesting an influence of specifically oestrogens on sperm transport. Oestrogens stimulate the synthesis and release of

PGF2α from the endometrium, which causes an increase in myometrial contractions (Claus, 1990). However, as was demonstrated by Weitze et al. (1989, 1990b), oestrogens do not seem to be the only factor in seminal plasma affecting sperm transport. Prior to artificial insemination with frozen semen (Weitze et al., 1989) or with semen containing $5x10^8$ sperm cells (Weitze et al., 1990b), gilts were pre-inseminated with seminal plasma, saline or saline with oestrogens. The number of accessory sperm cells in the zona pellucida was increased in gilts that were pre-inseminated with saline containing oestrogens, but was highest in the gilts that were pre-inseminated with seminal plasma. Viring and Einarsson (1980a) did not find an effect of seminal plasma on uterine motility, but found a decreased motility of the isthmus of the oviduct. The authors suggested that this relaxation facilitates the transport of sperm cells into the oviduct. This suggestion was confirmed in a sperm distribution study (Viring and Einarsson, 1980b). Therefore, besides uterine motility, also isthmic motility plays a role in sperm transport.

The volume of semen and the number of sperm cells inseminated may influence the accessory sperm count. Baker et al. (1968) inseminated gilts with $1x10^9$, $5x10^9$ or $10x10^9$ sperm cells in either 20, 100 or 200 ml of semen. At 12 hours after insemination (n=18; two gilts per experimental group), no accessory sperm cells were found in the 20 ml treatment, nor in the lowest doses in the 200 ml treatment. The highest dose combined with the largest volume gave the best results in terms of accessory sperm count. At 16 hours after insemination (n=27; three gilts per experimental group) no accessory sperm cells were found in gilts that had been inseminated with only $1x10^9$ sperm cells. The number of accessory sperm cells in the other treatments averaged from 7 ($5x10^9$-200 ml) to 387 ($10x10^9$-20 ml). The percentage of fertilized eggs was low (50% or less) in all $1x10^9$, all 20 ml and the $5x10^9$-200 ml treatments. At this stage (6 to 8 hours after ovulation) much variation exists in the relationship between the number of accessory sperm cells and the percentage of fertilized eggs. During natural mating, both the volume of seminal plasma and the number of sperm cells deposited in the sow may vary 2- to 3-fold, between 150 and 500 ml and between $15x10^9$ and $50x10^9$ cells, respectively (Pineda, 1989). Even although during natural mating the volume and the number of sperm cells are much higher than the levels used by Baker et al. (1968), differences in sperm transport and accessory sperm count between sows might consequently be expected.

From the above, it can be concluded that several boar stimuli, such as olfactory stimulation, tactile stimulation, sperm plasma volume and sperm plasma components such as oestrogens may influence transport of sperm to the site of fertilization. Both oxytocin and PGF2α levels may be influenced by boar stimuli. Both hormones are known to increase uterine motility, which is essential for sperm transport.

Ovulation Process

In animals such as the rabbit, cat, ferret and camel, mating causes an LH surge, subsequently causing (reflex) ovulation (McDonald, 1989). In a number of spontaneous ovulators, mating stimuli may influence ovulation-associated processes (Jöchle, 1975). Therefore the question arises whether or not in the pig (a spontaneous ovulator) mating stimuli also influence ovulation-associated processes.

LH release

Few researchers have studied effects of mating stimuli on LH release in the pig. Tilton et al. (1980) found a temporary stimulatory effect of mating on LH concentration at approximately one hour after copulation in sows in which LH levels were measured during 12 to 16 hours after onset of oestrus. Ziecik et al. (1980, 1981) reported a prolonged LH surge after natural mating compared to artificial insemination with whole semen, artificial insemination with seminal plasma and non mated controls. Kirsch et al. (1985) showed that both artificial insemination and natural mating hastened the maximal LH concentration compared to animals where the boar was allowed to mount but intromission was prevented. This suggests an influence of internal stimulation on LH release. However, the duration of the LH surge was less for the artificial insemination group than for the other two groups, also suggesting an influence of external boar stimuli on LH release. Total LH release during the surge was not influenced by the mating stimuli (Kirsch et al., 1985).

In general, some boar stimuli seem to be able to affect several aspects of LH release, e.g. the timing of the LH surge and the duration of the LH surge. In sows, the timing of the LH surge relative to the onset of oestrus may vary extremely (Tilton et al.,1982; Ziecik et al., 1982; Helmond et al., 1986), between 32 hours before to more than 24 hours after onset of oestrus. On average, the LH surge has been reported to occur at onset of oestrus (Niswender et al., 1970) and 10 h after onset of oestrus (Helmond et al., 1986). Under field conditions, therefore, the majority of sows is inseminated/mated after the LH surge. Therefore, effects of boar stimulation around mating on LH levels probably are limited to sows with a relatively late LH surge.

Ovulation

As early as 1951, Lebedev and Pitkjanen found an effect of mating stimuli on the process of ovulation in pigs. Sows were either not mated, mated once or mated twice, the latter with a mating interval of 5 to 10 minutes. At 28 hours after onset of oestrus, the number of sows that had ovulated was 'few', 70% and 'all', respectively. At 37 to 96 hours after service, within-litter embryonic development showed a greater uniformity, represented by a higher proportion of embryos at the 2 to 4 cell stage, in sows that were mated twice. Later

experiments showed that an additional mating with a vasectomized boar may also hasten the timing of ovulation in pigs (Pitkjanen, 1955). The timing of ovulation was not affected by double mating (onset of oestrus and 12 hours thereafter) compared to non-mating (Zimmerman and Naber, 1971). Signoret et al. (1972), however, also compared double mated gilts (onset of oestrus and 6 hours thereafter) with non-mated gilts and besides an earlier timing of ovulation (34.1 hours vs. 38.0 hours after onset of oestrus), they also found a shorter duration of ovulation (0.9 hours vs. 3.8 hours). More recently, Lotz et al (1989) and Weitze et al (1990a, c) showed that insemination with sperm plasma at the onset of oestrus may hasten ovulation with as much as 14 hours compared to non-inseminated controls. Furthermore, insemination with physiological saline or saline with oestrogens caused an intermediate timing of ovulation, suggesting that either vaginal/cervical stimulation or the presence of fluid in the uterus may also affect the ovulation process. Application of these treatments at 16 hours after onset of oestrus gave similar results except for physiological saline which did not influence the timing of ovulation (Weitze et al., 1990c; Willmen et al., 1991). The importance of sperm plasma in advancing ovulation was also demonstrated in an experiment in which pre-insemination of seminal plasma prior to insemination of $5x10^8$ sperm cells hastened ovulation compared to pre-insemination with oestrogens or an extender (Weitze et al., 1990b).

After insemination of a solution with oestrogens (naturally present in boar seminal plasma) at the first day of standing heat, Claus et al. (1987) found increased levels of both oestrogen- and PGF2α- in the uterine veins. They hypothesized that the oestrogens affect/advance the pre-ovulatory LH surge and that PGF2α affects the ovulation process directly at the ovaries. Ovulation is known to be associated with intra-follicular synthesis of PGF2α (reviewed by Ainsworth et al., 1990). Transcervical infusion of PGF2α led to an intra-follicular increase of PGF2α-concentration and also advanced ovulation by approximately 12 hours (Weiler and Claus, 1991). However, results found by Weitze et al. (1990a, b, c) as referred to above, make clear that oestrogens are not the only factor in seminal plasma advancing ovulation, since ovulation was more advanced after seminal plasma application. At this moment, it is not clear which factor(s) is(are) responsible for this effect and whether or not the(their) mechanism(s) of action on the process of ovulation is similar to that of oestrogens.

Reproductive Performance

Boar stimuli at or around insemination may affect reproductive characteristics such as standing response, sperm transport and process of ovulation. The question arises whether or not these effects are of importance for reproductive performance, not only during pregnancy (fertilization rate, embryonic development) but also at farrowing (farrowing rate, litter size). Therefore, effects of boar stimulation at or around insemination on reproductive performance

during early pregnancy and at farrowing will be summarized.

Fertilization Rate and Embryonic Development

Little is known about effects of mating stimuli on fertilization rate and embryonic development. Kruff et al. (1983) compared double natural mating with double artificial insemination and found that, at Day 4 of pregnancy, embryos of the naturally mated gilts were further developed ($P < 0.001$), suggesting that boar stimulation affected either the rate of early embryonic development or the timing of ovulation. The latter would correspond with results of Signoret et al. (1972) and Weitze et al. (1990a, c) as described before. In sows that were mated twice at an interval of 5 to 10 minutes, within-litter embryonic development at 37 to 96 hours after service showed a greater uniformity than in sows that were mated once (Lebedev and Pitkjanen, 1951). This suggests that boar stimulation affected either the duration of ovulation as reported by Signoret et al. (1972) or the within-litter rate of early embryonic development.

A specific mating stimulus, seminal plasma, has been found to affect both fertilization rate and embryonic development. Weitze et al. (1989) showed that insemination of seminal plasma immediately prior to insemination of frozen semen increased fertilization rate determined after 3-5 Days (82.8%) compared with infusion of saline containing oestrogens (56.7%) and saline (36.4%). Similar treatments applied prior to insemination of liquid semen containing $5x10^8$ sperm cells resulted in fertilization rates of 85.2, 74.5 and 53.6, respectively (all contrasts $P < 0.05$) (Weitze et al., 1990b). The described effects on fertilization rate might be related to a better timing between insemination and ovulation due to increased sperm transport and advanced ovulation in sows that were treated with seminal plasma. Rath et al. (1989) showed that pre-insemination with seminal plasma compared to pre-insemination with a commercial BSA diluter, both followed by artificial insemination with $2x10^9$ sperm cells, increased the percentage of normally developed (= fertilized and non-degenerated) embryos after 3 to 5 Days (92.5% vs 75.8%, $P < 0.001$). This difference in percentage of normally developed embryos is due to non-significant differences in fertilization rate and degeneration rate. In this experiment, the animals were inseminated 24 hours after detected onset of oestrus (twice daily oestrus detection), while the duration of oestrus was relatively short, ranging from 36 to 60 hours. The better results after pre-insemination with sperm plasma, despite the short interval between insemination and ovulation, might be due to an advanced sperm transport (accessory sperm count increased from 25.3 to 85.2; $P < 0.05$) rather than an advanced ovulation. It is known that late insemination involves both decreased fertilization rates and increased embryonic mortality rates due to aging of oocytes before fertilization (Helmond et al., 1986).

Pregnancy Rate and Litter Size

Drugociu (1966) studied the effect of a number of insemination conditions on reproductive performance and found that farrowing rates and, to a lesser extent, litter sizes were highest in sows that had been housed with an aproned boar before service, indicating the importance of the external boar stimuli on reproductive processes. Hemsworth et al. (1978) found a significant correlation ($r=0.48$, $P<0.05$) between the number of times a boar noses the sides of a sow and subsequent reproductive performance in terms of pregnancy rate. This is an indication of the importance of the courting behaviour before mating on reproductive processes. Furthermore, they found that two minutes contact with a boar before artificial insemination significantly affected litter size.

In a number of experiments, vasectomized boars have been used for additional boar stimuli around insemination. The results are inconsistent. In embryo-transfer trials performed by Fenton et al. (1972), mating of the recipients by a vasectomized boar during oestrus resulted in an increase in pregnancy rate at Day 25 after oestrus (6/8 vs 2/9). If repeatable, these results might suggest an effect of mating on the uterine environment. Furthermore, Mah et al. (1985) found that mating with a vasectomized boar immediately after each mating (12 and 24 hours after onset of oestrus) increased conception rates at Day 30 of pregnancy from 84% ($n=45$) to 100% ($n=42$). The number of embryos and the number of corpora lutea were unaffected. In a field trial, Davies (1986) found no effect on farrowing rate (86% vs 81%), but an increased litter size (11.2 vs 12.2; $P<0.05$) when sows were housed with a vasectomized boar for the 24 hours between two matings with a fertile boar. Mating with a vasectomized boar ($n=78$) immediately after artificial insemination with $3\text{-}6\times10^9$ frozen sperm cells at 10-16 hours after onset of oestrus and 24 hours thereafter, failed to improve the low farrowing rates and litter sizes obtained (38.4% vs 37.2% and 7.5 vs 7.2, respectively; $n=164$) in gilts (Pursel et al., 1982). Based on results of Baker et al. (1968), the authors hypothesised that dilution of the sperm cells by sperm plasma precluded possible positive effects of the additional matings.

The specific effect of oestrogens on pregnancy rate, embryonic mortality and litter size was investigated in a number of experiments. The results are inconsistent. Addition of oestrogens (11.5 μg) to AI-doses applied at 16 and 32 hours after onset of oestrus compared to control AI failed to affect embryonic survival (79 ± 4% vs 74 ± 4%) and pregnancy rate (80% vs 85%) at Day 25 of pregnancy (Lambert et al., 1991). Similarly, addition of seminal plasma to frozen boar sperm failed to affect pregnancy rate (73% vs 73%) and number of embryos (13.2 ± 6.6 vs 10.8 ± 5.6) at Day 28 of pregnancy (Weitze et al., 1991). Kirkwood and Thacker (1991) added 10 μg oestrogens to AI-doses (1-3 times per oestrus at 12 hours intervals) and found a significant increase in pregnancy rate (78% vs 90%; $P<0.10$) at Day 85 of pregnancy. In a second trial in which two inseminations were applied at 24 hours intervals, oestrogens failed to improve farrowing rate (80% vs 85%; n.s.) and litter size

(10.8±0.4 vs 11.3±0.5; n.s.) (Kirkwood and Thacker, 1991). However, in a field trial, Claus et al. (1989a) found improved farrowing rates (77% vs 83%; $P<0.05$) and litter sizes (10.3 vs 10.8; $P<0.05$) in sows in which 11.5 μg oestrogens were added to the AI-doses. It can be concluded that effects of oestrogens in the inseminate on reproductive performance are very variable (Claus et al., 1989a; Kirkwood and Thacker, 1991; Lambert et al., 1991). At present, the causes of this variability are not known.

BOAR STIMULATION: INTERACTION WITH SOCIAL CONDITIONS

Effects of boar stimulation at or around insemination on reproductive characteristics of sows as presented, are not independent of other factors. In this paragraph, the influence of the social conditions of sows on these phenomena and their possible mechanism of action will be discussed.

Many experiments have been performed in which the effects were studied of social conditions (individual, group, confined, tethered) on reproductive parameters such as puberty attainment (Dyck, 1988; Prunier and Meunier-Salaün, 1989), weaning to mating interval (Hemsworth et al., 1982b), farrowing rate (Jensen et al, 1970; Barnett and Hemsworth, 1991) and litter size (England and Spurr, 1969) and also effects of social conditions on the existence and/or extent of a stress response (Barnett et al., 1981, 1986, 1987; Becker et al., 1984, 1985; Rampacek et al., 1984). Although not all experiments showed significant effects, restricted housing conditions, concerning e.g social conditions, tethering or restriction of space, in general negatively affect reproductive parameters, possibly due to a stress response (Coubrough, 1985).

Social conditions of sows may, furthermore, affect sexual behaviour. Social restriction of gilts during rearing and social restriction of cycling gilts and sows during pregnancy have been found to negatively affect subsequent sexual behaviour concerning e.g. standing response latency, restlessness during copulation and percentage of successful copulations (Hemsworth et al., 1982a, 1986; Barnett and Hemsworth, 1991).

Stress conditions have been found to influence for example embryonic mortality and conception rate, the extent of influence depending on the stressor involved, the duration of the stress or the intensity or strength of the stressor (reviewed by Coubrough, 1985). The overriding effect of stress on reproductive functions lies in the neuro-endocrine system; several products of the hypothalamic-pituitary-adrenal (HPA) axis have been recognized to interfere with all three levels of the hypothalamic-pituitary-gonadal (HPG) axis, especially during prolonged stress. Also evidence is accumulating that the influence of both prolonged stress and acute stress on reproductive function is mediated by a variety of neurotransmitters acting within the brain (reviewed by Rivier and Rivest, 1991).

The importance of social conditions of sows when studying influences of boar stimulation, was shown by Hemsworth et al. (1978). They found a positive effect of boar contact before artificial insemination on litter size in pairwise housed sows, whereas litter size was decreased after comparable boar contact in individually housed sows. The differences in pregnancy rate showed the same tendencies, but were not significant. These differences in litter size and pregnancy rate might be a consequence of differences in housing conditions. Barnett et al (1982) showed that in individually housed sows (chronically stressed according to Barnett et al. (1981)) compared to pairwise housed sows, a weakened response to acute stressors like mating exists. They hypothesized that a high level of corticosteroids after mating (as measured in the pairwise housed gilts) may enhance the LH release, stimulate the process of ovulation and subsequently affect fertility positively.

From the above it is clear that, although boar stimuli around insemination may affect reproductive performance in a positive way, the effects may also be negative, depending on the social (housing) conditions of the sow. No information is available concerning the interaction between the effect of social conditions and the effect of boar stimuli on early pregnancy.

IN CONCLUSION

In pig husbandry, reproductive performance after either natural mating or artificial insemination is considered to be similar. However, possible differences in reproductive performance have hardly been evaluated experimentally. One experimental comparison, performed in the U.S.A. by Hagen (1986 in Crabo, 1990), did not show any differences in farrowing rate or litter size. In the present review, however, abundant evidence is presented that specific boar stimuli may affect reproductive processes in oestrous sows. In general, stimulation of reproductive processes by boars may be of particular importance when conditions such as the timing of insemination (due to suboptimal oestrus detection), the number of inseminated sperm cells and/or the inseminated volume are suboptimal (e.g. Baker et al., 1968; Weitze et al., 1989; Weitze et al., 1990b). External stimulation, such as olfactory and tactile stimulation, evokes the standing response and stimulates sperm transport through increased oxytocin levels. Internal stimulation, such as tactile stimulation, sperm plasma and seminal oestrogens, stimulates sperm transport by increased oxytocin levels and increased PGF2α-levels and the process of ovulation through increased PGF2α-levels.

Little is known concerning the influence of oxytocin on the process of ovulation. In sheep, follicular oxytocin production starts during the LH surge, increases slightly up to ovulation and reaches its maximum in the young corpus luteum (Wathes et al., 1986). In cattle, pre-ovulatory follicles produce oxytocin (Voss and Fortune, 1991) and transcription of

the oxytocin gene is maximal during ovulation (Ivell et al., 1985). In humans, oxytocin is produced by pre-ovulatory follicles and inhibits collagen synthesis and, therefore, is assumed to participate in the regulation of follicular rupture (Tjugum et al., 1986). If oxytocin affects the process of ovulation in pigs similarly, it might be hypothesized that external boar stimulation not only evokes the standing response and stimulates sperm transport, but may also stimulate the process of ovulation. Thus, the results of Hemsworth et al. (1978) concerning the positive influence of courting and nosing activity on reproductive performance might be explained by both a stimulated sperm transport and a stimulated ovulation process. Synchronization of sperm transport and the ovulation process synchronizes the presence of fertile male and female gametes in the oviduct, consequently increasing the chance of fertilization, pregnancy rate and litter size. Concerning the process of ovulation, both the timing and duration of ovulation may be influenced by boar stimulation. The duration of ovulation is of importance because of its hypothesised negative relation with litter size. A longer duration of ovulation causes an increase in within-litter embryonic diversity (Pope et al., 1988; Xie et al., 1990), which in turn increases the chance of embryonic mortality (reviewed by Pope et al., 1990) and thus decreases litter size. Boar stimulation may influence this mechanism as was shown by its effect on the duration of ovulation (Signoret et al., 1972), within-litter embryonic diversity (Lebedev and Pitkjanen, 1951) and litter size (Hemsworth et al., 1978).

Inconsistent results between experiments as described in the present review, may be due to individual differences between animals in genetic background, ontogeny and later experience, which are known to influence the physiology and behaviour of animals (Levine et al., 1989). As an example, social conditions of sows may influence effects of boar stimulation on reproductive characteristics. Both sexual behaviour and, through (neuro-)endocrine changes, the process of ovulation may be affected. These variable effects of boar stimulation depending on social conditions may furthermore (partly) explain the lack of difference found between artificial insemination and natural mating concerning reproductive performance under field conditions.

Based on the evidence presented, the use of for example vasectomized boars for additional boar stimulation around insemination may be worthwhile, despite the reduced level of seminal oestrogens in vasectomized boars (Claus et al., 1987). Although effects of seminal oestrogens on sperm transport and the process of ovulation seem consistent (e.g. Claus, 1990; Weitze et al., 1990c) effects on reproductive performance are variable (Claus, 1989a; Kirkwood and Thacker, 1991; Lambert et al., 1991) and need further investigation. Despite variable results under experimental conditions, the addition of hormones such as oestrogens and oxytocin to the inseminate could improve reproductive performance after artificial insemination, because of their stimulating effects on sperm transport and the process of ovulation. These hormones may, furthermore, assist ova pick-up from the ovary through

increased muscular activity of the ligamentum infundibulo-cornuale (Persson and Rodriquez-Martinez, 1990).

Many aspects of boar stimuli, including effects and mechanisms have not been clarified yet. A better understanding of these effects and mechanisms, taking into account the (social) background of sows, might lead to new strategies to improve reproductive performance.

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REFERENCES

Ainsworth, L., Tsang, B.K., Downey, B.R. and Marcus, G.J., 1990. The synthesis and actions of steroids and prostaglandins during follicular maturation in the pig. J. Reprod. Fertil., Suppl. 40: 137-150.

Baker, R.D., Dziuk, P.J. and Norton, H.W., 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. J. Anim. Sci., 27: 88-93.

Barnett, J.L. and Hemsworth, P.H., 1991. The effects of individual and group housing on sexual behaviour and pregnancy in pigs. Anim. Reprod. Sci., 25: 265-273.

Barnett, J.L., Cronin, G.M. and Winfield, C.G., 1981. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. Gen. Comp. Endocrinol., 44: 219-225.

Barnett, J.L., Hemsworth, P.H. and Cronin, G.M., 1982. The effect of mating on plasma corticosteroids in the female pig and the influence of individual and group penning on this response. Gen. Comp. Endocrinol., 47: 516-521.

Barnett, J.L., Hemsworth, P.H., Winfield, C.G. and Hansen, C., 1986. Effects of social environment on welfare status and sexual behaviour of female pigs. I. Effects of group size. Appl. Anim. Behav. Sci., 16: 249-257.

Barnett, J.L., Hemsworth, P.H., Winfield, C.G. and Fahy, V.A., 1987. The effects of pregnancy and parity number on behavioral and physiological responses related to the welfare status of individual and group-housed pigs. Appl. Anim. Behav. Sci., 17: 229-243.

Becker, B., Christenson, R.K., Ford, J., Manak, R., Nienaber, J., Hahn, G. and Deshazer, J., 1984. Serum cortisol concentrations in gilts and sows housed in tether stalls, gestation stalls and individual pens. Ann. Rech. Vét., 15: 237-242.

Becker, B.A., Ford, J.J., Christenson, R.K., Manak, R.C., Hahn, G.L. and DeShazer, J.A., 1985. Cortisol response of gilts in tether stalls. J. Anim. Sci., 60: 264-270.

Blandau, R.J. and Gaddum-Rosse, P., 1974. Mechanism of sperm transport in pig oviducts. Fertil. Steril., 25: 61-67.

Booth, W.D., 1988. Hormones, pheromones and sexual behaviour in the boar. Pig News Inf., 9: 251-255.

Claus, R., 1990. Physiological role of seminal components in the reproductive tract of the female pig. J. Reprod. Fertil., Suppl. 40: 117-131.

Claus, R., Hoang-Vu, C., Ellendorff, F., Meyer, H.D., Schopper, D. and Weiler, U., 1987. Seminal oestrogens in the boar: origin and functions in the sow. J. Steroid Biochem., 27: 331-335.

Claus, R., Moshammer, T., Aumüller, R. and Weiler, U., 1989a. Replenishment of AI-doses with oestrogens in physiological amounts: effect on sow prolificacy in a field trial. J. Vet. Med. A, 36: 797-800.

Claus, R., Ellendorff, F. and Hoang-Vu, C., 1989b. Spontaneous electromyographic activity throughout the cycle in the sow and its change by intrauterine oestrogen infusion during oestrus. J. Reprod. Fertil., 87: 543-551.

Coubrough, R.I., 1985. Stress and fertility. A review. Onderstepoort J. Vet. Res., 52: 153-156.

Crabo, B.G., 1990. Preservation of boar semen: a worldwide perspective. Reprod. Dom. Anim., Suppl.1: 3-9.

Davies, O.D., 1986. Effect of using vasectomized boars for additional matings on sow reproductive performance. Brit. Soc. Anim. Prod., Wintermeeting Scarborough. Paper no. 123, 2pp.

Döcke, F. and Worch, H., 1963. Untersuchungen über die Uterusmotilität und die Paarungsreaktionen der Sau. Zuchthygiene, 7: 169-178.

Drugociu, G., 1966. The importance of internal and external stimuli for sexual functions in the sow. Anim. Breed. Abstr., 36(1968): 276 (Abstract).

Dyck, G.W., 1988. Factors influencing sexual maturation, puberty and reproductive efficiency in the gilt. Can. J. Anim. Sci., 68: 1-13.

England, D.C. and Spurr, D.T., 1969. Litter size of swine confined during gestation. J. Anim. Sci., 28: 220-

223.

Fenton, F.R., Schwartz, F.L., Bazer, F.W., Robison, O.W. and Ulberg, L.C., 1972. Stage of gestation when uterine capacity limits embryo survival in gilts. J. Anim. Sci., 35: 383-388.

Fléchon, J.E. and Hunter, R.H.F., 1981. Distribution of spermatozoa in the utero-tubal junction and isthmus of pigs, and their relationship with the luminal epithelium after mating: a scanning electron microscope study. Tissue and Cell, 13: 127-139.

Hawk, H.W., Sperm survival and transport in the female reproductive tract. J. Dairy Sci., 66: 2645-2660.

Helmond, F., Aarnink, A. and Oudenaarden, C., 1986. Periovulatory hormone profiles in relation to embryonic development and mortality in pigs. In: J.M.Sreenan and M.G.Diskin (Editors). Embryonic mortality in farm animals. Martinus Nijhoff Publishers, Dordrecht, pp. 119-125.

Hemsworth, P.H. and Barnett, J.L., 1990. Behavioral responses affecting gilt and sow reproduction. J. Reprod. Fertil., Suppl. 40: 343-354.

Hemsworth, P.H., Beilharz, R.G. and Brown, W.J., 1978. The importance of the courting behaviour of the boar on the success of natural and artificial matings. Appl. Anim. Ethol., 4: 341-347.

Hemsworth, P.H., Cronin, G.M. and Hansen, C., 1982a. The influence of social restriction during rearing on the sexual behaviour of the gilt. Anim. Prod., 35: 35-40.

Hemsworth, P.H., Salden, N.T.C.J. and Hoogerbrugge, A., 1982b. The influence of the post-weaning social environment on the weaning to mating interval of the sow. Anim. Prod., 35: 41-48.

Hemsworth, P.H., Barnett, J.L., Hansen, C. and Winfield, C.G., 1986. Effects of social environment on welfare status and sexual behaviour of female pigs. II. Effects of space allowance. Appl. Anim. Behav. Sci., 16: 259-267.

Hemsworth, P.H., Winfield, C.G., Tilbrook, A.J., Hansen. C. and Barnett, J.L., 1988. Habituation to boar stimuli: possible mechanism responsible for the reduced detection rate of oestrous gilts housed adjacent to boars. Appl. Anim. Behav. Sci., 19: 255-264.

Hughes, P.E., Hemsworth, P.H. and Hansen, C., 1985. The effects of supplementary olfactory and auditory stimuli on the stimulus value and mating success of the young boar. Appl. Anim. Behav. Sci., 14: 245-252.

Hughes, P.E., Pearce, G.P. and Patterson, A.M., 1990. Mechanisms mediating the stimulatory effects of the boar on gilt reproduction. J. Reprod. Fertil., Suppl. 40: 323-341.

Hunter, R.H.F., Cook, B. and Poyser, N.L., 1983. Regulation of oviduct function in pigs by local transfer of ovarian steroids and prostaglandins: a mechanism to influence sperm transport. Europ. J. Obstet. Gynec. Reprod. Biol., 14: 225-232.

Ivell, R., Brackett, K.H., Fields, M.J. and Richter, D., 1985. Ovulation triggers oxytocin gene expression in the bovine ovary. FEBS-Lett. 190: 263-267.

Jensen, A.H., Yen, J.T., Gehring, M.M., Baker, D.H., Becker, D.E. and Harmon, B.G., 1970. Effects of space restriction and management on pre- and post-puberal response of female swine. J. Anim. Sci., 31: 745-750.

Jöchle, W., 1975. Current research in coitus-induced ovulation: a review. J. Reprod. Fertil., Suppl. 22: 165-207.

Kirkwood, R. and Thacker, P.A., 1991. The influence of adding oestradiol to semen on reproductive performance of sows., Can. J. Anim. Sci., 71: 589-591.

Kirsch, J.D., Tilton, J.E., Ziecik, A., Weigl, R., Schaffer, T. and Williams, G.L., 1985. Effects of various mating stimuli on pituitary release of luteinizing hormone in the gilt. Dom. Anim. Endocrinol., 2: 99-104.

Krajnák, P., 1988. The effect of oxytocin added to insemination doses on fertility in pigs. Pig News Inf., 11 (1990): 388 (Abstract).

Kruff,B., Lampeter, W.W. and Kräusslich, H., 1983. Untersuchungen zum Superovulationserfolg und zur Kultur von Embryonen im Rahmen des Embryotransfers beim Schwein. Zuchthygiene, 18: 1-6.

Lambert, E., Williams, D.H., Lynch, P.B., Hanrahan, T.J., McGeady, T.A., Austin, F.H., Boland, M.P. and

Roche, J.F., 1991. The effects of administration of gonadotrophins and estrogens on prenatal survival in gilts. Theriogenology, 36: 77-85.

Lebedev, M.M. and Pitkjanen, I.M., 1951. Increasing fertility in pigs. Anim. Breed. Abstr., 20 (1952): 57 (Abstract).

Levine, S., Coe, C. and Wiener, S.G., 1989. Psychoneuroendocrinology of stress: a psychobiological perspective. In: F.R. Brush and S. Levine (Editors). Psychoendocrinology. Academic Press, Inc., San Diego, USA, pp. 341-377.

Lotz, J., Weitze, K.F., Rabeler, J., Willmen, T. and Waberski, D., 1989. Influence of intracervical infusion of seminal plasma, oestrogens and physiological saline on ovulation time in gilts as studied by transcutaneous sonography. Third Intern. Confer. on Pig Reprod., Nottingham, UK. Abstract No. 41.

Mah, J., Tilton, J.E., Williams, G.L., Johnson, J.N. and Marcello, M.J., 1985. The effect of repeated mating at short intervals on reproductive performance of gilts. J. Anim. Sci., 60: 1052-1054.

Mattioli, M., Galeati, G., Conte, F. and Seren, E., 1986. Effect of 5α-androst-16-en-3-one on oxytocin release in oestrous sows. Theriogenology, 25: 399-403.

McDonald, L.E., 1989. Patterns of reproduction. In: L.E. McDonald (Editor). Veterinary endocrinology and reproduction. Lea and Febiger, Philadelphia, USA, pp. 389-398.

Niswender, G.D., Reichert, L.E. and Zimmerman, D.R., 1970. Radioimmunoassay of serum levels of luteinizing hormone throughout the estrous cycle in pigs. Endocrinology, 87: 576-580.

Odehnal, F., Barth, T. and Jost, K., 1989. The effect of Depotocin^{R} (carbetocin) added to insemination doses of boar semen on the conception of sows and their fertility. Pig News Inf., 11 (1990): 203 (Abstract).

Perry, G.C., 1982. The role of olfaction in the reproductive behaviour of pigs. Pig News and Information, 3: 11-15.

Perry, G.C., Patterson, R.L.S., MacFie, H.J.H. and Stinson, C.G., 1980. Pig courtship behaviour: pheromonal property of androstene steroids in male submaxillary secretion. Anim. Prod., 31:191-199.

Persson, E. and Rodriquez-Martinez, H., 1990. The ligamentum infundibulo-cornuale in the pig: morphological and physiological studies of the smooth muscle component. Acta Anat. Basel, 138: 111-120.

Pineda, M.H., 1989. Male reproduction. In: L.E. McDonald (editor). Veterinary endocrinology and reproduction. Lea and Febiger, Philadelphia, USA, pp.261-303.

Pitkjanen, I.G., 1955. Ovulation, Fertilisation and the first stages of embryonic development in pigs. Anim. Breed. Abstr., 24(1956): 51.

Pope, W.F., Wilde, M.H. and Xie, S., 1988. Effect of electrocautery of nonovulated Day 1 follicles on subsequent morphological variation among Day 11 porcine embryos. Biol. Reprod., 39: 882-887.

Pope, W.F., Xie, S., Broermann, D.M. and Nephew, K.P., 1990. Causes and consequences of early embryonic diversity in pigs. J. Reprod. Fertil., Suppl.40: 251-260.

Prunier, A. and Meunier-Salaün, M.C., 1989. The influence of boar stimulation on puberty attainment in tethered and group penned gilts. Theriogenology, 32: 949-959.

Pursel, V.G., Elliot, D.O. and Newman, C.W., 1982. Mating by vasectomized boars failed to improve fertility in gilts inseminated with frozen sperm. Theriogenology, 18: 61-64.

Rampacek, G.B., Kraeling, R.R., Fonda, E.S. and Barb, C.R., 1984. Comparison of physiological indicators of chronic stress in confined and nonconfined gilts. J. Anim. Sci., 58: 401-408.

Rath, D., Weitze, K.F., Pena Alfaro, C.E. and Andrade Moura, J.C., 1989. Effects of seminal plasma on the number of accessory sperm cells and fertilization in gilts. Zuchthygiene, 24: 123-127.

Reeves, J.J., 1987. Endocrinology of Reproduction. In: E.S.E. Hafez (Editor). Reproduction in farm animals. Lea and Febiger, Philadelphia, USA, pp. 85-106.

Rivier, C. and Rivest, S., 1991. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biol. Reprod., 45: 523-532.

Schenk, P.M., 1967. An investigation into the oestrus symptoms and behaviour of sows. Z. Tierzücht und Züchtgsbiol., 83: 87-110.

Sergeev, N.I., 1963. Results of artificially inseminating pigs when oxytocin and neurotropic substances were used. Anim. Breed. Abstr., 31(1963): 388 (Abstract).

Signoret, J.P., 1970. Reproductive behaviour of pigs. J. Reprod. Fert., Suppl. 11: 105-117.

Signoret, J.P., 1971a. The mating behaviour of the sow. In: D.J.A. Cole (Editor). Pig Production. Butterworths, London, UK, pp. 295-313.

Signoret, J.P., 1971b. The reproductive behaviour of pigs in relation to fertility. Vet. Rec., 88: 34-38.

Signoret, J.P. and Bariteau, J., 1975. Utilisation de différents produits odorants de synthèse pour faciliter la détection des chaleurs chez la truie. Ann. Zootech., 24: 639-643.

Signoret, J.P., du Mesnil du Buisson, F. and Mauléon, P., 1972. Effect of mating on the onset and duration of ovulation in the sow. J.Reprod. Fertil., 31: 327-330.

Tanida, H., Miyazaki, N., Tanaka, T. and Yoshimoto, T., 1991. Selection of mating partners in boars and sows under multi-sire mating. Appl. Anim. Behav. Sci., 32: 13-21.

Tilton, J.E., Ziecik, A., Kirsch, J.D. and Weigl, R., 1980. Patterns of the LH release in pigs during oestrus. J. Anim. Sci., 51(Suppl 1): 89-90.

Tilton, J.E., Foxcroft, G.R., Ziecik, A.J., Coombs, S.L. and Williams, G.L., 1982. Time of the preovulatory LH surge in the gilt and sow relative to the onset of behavioral estrus. Theriogenology, 18: 227-236.

Tjugum, J., Norstrom, A., Dennefors, B. and Lundin, S., 1986. Oxytocin in human follicular fluid and its possible role in the ovulatory process as studied in vitro. Hum. Reprod., 1: 283-286.

Viring, S. and Einarsson, S., 1980a. Effect of boar seminal plasma on uterine and oviductal motility in oestrous gilts. Acta Vet. Scand., 21: 607-616.

Viring, S. and Einarsson, S., 1980b. Influence of boar seminal plasma on the distribution of spermatozoa in the genital tract of gilts. Acta Vet. Scand., 21: 598-606.

Voss, A.K. and Fortune, J.E., 1991. Oxytocin secretion by bovine granulosa cells: effects of stage of follicular development, gonadotropins, and coculture with theca interna. Endocrinology, 128: 1991-1999.

Wathes, D.C., Guldenaar, S.E., Swann, R.W., Webb, R., Porter, D.G. and Pickering, B.T., 1986. A combined radioimmunoassay and immunocytochemical study of ovarian oxytocin production during the periovulatory period in the ewe. J. Reprod. Fertil., 78: 167-183.

Weiler, U. and Claus, R., 1991. Endocrine aspects of testicular function, especially hormones in the seminal plasma and their fate in the female reproductive tract: testicular steroids and their relevance for male and female reproductive functions. Reprod. Dom. Anim., Suppl.1: 41-61.

Weitze, K.F., Rath, D. and Andrade Moura, J.C., 1989. Spermientransport und Befruchtungsergebnisse nach Besamung mit Gefriersperma beim Schwein. Zuchthygiene, 24: 223-228.

Weitze, K.F., Rath, D., Willmen, T., Waberski, D. and Lotz, J., 1990a. Advancement of ovulation in the sow related to seminal plasma application before insemination., Reprod. Dom. Anim., 25: 61-67.

Weitze, K.F., Rabeler, J., Willmen, T. and Waberski, D., 1990b. Interaction between inseminate, uterine and ovarial function in the sow. I. Influence of seminal plasma and oestrogens in the inseminate on intragenital sperm transport, time of ovulation and fertility results in gilts. Reprod. Dom. Anim. 25: 191-196.

Weitze, K.F., Lotz, J., Everwand, A., Willmen, T. and Waberski, D., 1990c. Interaction between inseminate, uterine and ovarial function in the sow II. Investigations into the influencing of ovulation by the use of sperm free media. Reprod. Dom. Anim., 25: 197-204.

Weitze, K.F., Stampa, E., Richter, L., Willmen, T. and Waberski, D., 1991. Fertility of frozen boar semen: influence of packaging, number of inseminations, and seminal plasma. Reprod. Dom. Anim., Suppl. 1: 139-142.

Willemse, A.H. and Boender, J., 1966. A quantitative and qualitative analysis of oestrus in gilts. Tijdschr. Diergeneesk., 91: 349-363.

Willmen, T., Rabeler, J., Everwand, A., Waberski, D. and Weitze, K.F., 1991. Influence of seminal plasma and oestrogens in the inseminate on fertilization rate, sperm transport, and ovulation time. Reprod. Dom.

Anim., Suppl. 1: 379-383.

Wodzicka-Tomaszewska, M., Kilgour, R. and Ryan, M., 1981. "Libido" in the larger farm animals: a review. Appl. Anim. Ethol., 7: 203-238.

Xie, S., Broermann, D.M., Nephew, K.P., Geisert, R.D. and Pope, W.F., 1990. Ovulation and early embryogenesis in swine. Biol. Reprod., 43: 236-240.

Ziecik, A., Tilton, J.E. and Weigl, R., 1980. Effect of mating on LH surge in pigs. J. Anim. Sci., 51(Suppl 1): 90.

Ziecik, A., Tilton, J.E. and Williams, G.L., 1981. Effect of mating on the luteinizing hormone surge in the pig. J. Anim. Sci., 53: 434-438.

Ziecik, A., Krzymowska, H. and Tilton, J.E., 1982. Porcine LH levels during the estrus cycle, gestation, parturition and early lactation. J. Anim. Sci., 54: 1221-1225.

Zimmerman, D.R. and Naber, C., 1971. Influence of mating on ovulation time in the pig. J. Anim. Sci., 31: 273.

Chapter 2

EFFECT OF INSEMINATION CONDITIONS ON REPRODUCTIVE PERFORMANCE DURING EARLY PREGNANCY OF GILTS KEPT UNDER DIFFERENT SOCIAL CONDITIONS

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EFFECT OF INSEMINATION CONDITIONS ON REPRODUCTIVE PERFORMANCE DURING EARLY PREGNANCY OF GILTS KEPT UNDER DIFFERENT SOCIAL CONDITIONS

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ABSTRACT

The effect of insemination conditions (artificial insemination vs artificial insemination followed by 20 min intensive boar contact) on reproductive performance during early pregnancy was studied in each of two groups of gilts. These groups were held under different social conditions from an age of approximately 137 days onwards; 30 were housed individually without boar contact (group 1), and 36 were housed pairwise, having daily boar contact from approximately 180 days of age onwards (group 2). All gilts were inseminated at third oestrus and slaughtered on Day 10 ± 1 after insemination.

Boar contact after insemination did not affect pregnancy rate, average embryonic mortality rate or average embryonic development in either of the two groups. In the gilts that had no boar contact after insemination, a positive relationship between age or weight at insemination and embryonic mortality was found.

Social conditions affected reproductive performance. In group 1, fewer gilts reached puberty spontaneously before 250 days of age in comparison with group 2 (15/30 vs 28/36) and the remaining gilts in group 1 responded less to oestrus induction with PG600 compared to those in group 2 (6/15 did not respond vs 0/8). Pregnancy rate was also adversely affected in group 1, whereas embryonic mortality was unaffected. Uterine development was not affected by insemination inseminations or by social conditions of the gilts either.

The results indicate that boar stimuli after insemination affected the relationship between age or weight at insemination and embryonic mortality rate in both social groups identically, but did not affect pregnancy rate, average embryonic mortality rate or embryonic development around Day 10 of pregnancy in either of the two social groups.

Keywords: social conditions; insemination conditions; gilt; early pregnancy.

INTRODUCTION

During oestrus the physical, auditory, visual and olfactory stimuli of the boar are necessary to elicit an optimal standing response in sows (Signoret, 1970). Similar stimuli during mating seem to influence reproductive performance: Hemsworth et al. (1978) found a significantly positive correlation between the number of times a boar nosed the sides of a sow

around mating and subsequent conception rate, and Mah et al. (1985) found a positive effect of post-insemination mating by a vasectomized boar on conception rate. Evidence is accumulating that boar stimuli not only may cause advanced sperm transport (Dőcke and Worch, 1963; Viring and Einarsson, 1980a, b; Mattioli et al., 1986), but may also influence processes that affect the timing of ovulation and/or the ovulation interval (Signoret et al., 1972; Tilton et al., 1980; Ziecik et al., 1981; Kirsch et al., 1985; Claus et al., 1987; Weitze et al., 1990).

Social conditions such as boar contact, individual vs. group housing, group size, human handling, space allowance and confinement, have an effect on reproductive parameters such as the attainment of puberty, sexual behaviour, litter size, weaning to oestrus interval and associated changes in levels of gonadotrophic hormones and corticosteroids (England and Spurr, 1969; Sommer, 1980; Barnett et al., 1982; Hemsworth et al., 1982a, b; Kirkwood and Hughes, 1982; Booth, 1984; Barnett et al., 1986; Hemsworth et al., 1986; Paterson et al., 1987; Hughes et al., 1990).

Besides the fact that insemination conditions and social conditions may influence reproductive performance separately, Hemsworth et al. (1978) found that the effect of insemination conditions on reproductive performance depends on the social conditions of the sows.

Embryonic mortality is a major cause of variation in litter size and therefore early pregnancy (the embryonic phase) has been the subject of many studies, as has been recently reviewed by Pope and First (1985) and Dziuk (1987). So far, however, the influence of insemination conditions on early pregnancy has not been investigated. In the present study, the effect of insemination conditions (artificial insemination vs artificial insemination followed by intensive contact with a vasectomized boar) on reproductive performance, with special emphasis on early pregnancy, was studied in each of two groups of gilts, differing in social environment.

MATERIALS AND METHODS

Animals

A total of 66 prepuberal Great Yorkshire x British Landrace (GYxBL) gilts (Pig Improvement Company, UK) were used, being 127 to 149 (137.4 ± 6.3) days of age upon arrival at the experimental farm. Rearing conditions before arrival are unknown.

Housing and Feeding

At arrival, 30 gilts were housed in individual pens (3.1 m^2) which did not allow tactile and visual contact with other gilts. The remaining 36 gilts were housed in pairs (6.2 m^2/pen)

and had additional visual and tactile contact with gilts in adjacent pens. From approximately 180 days of age, one of three vasectomized boars was introduced alternately once daily to the pairwise housed gilts (approximately 10 a.m., about 1 min per pen), to increase social differences between the two groups of gilts. All other environmental conditions were similar in both groups.

Gilts were weighed weekly and fed a commercial sow ration (12.18 MJ ME per kg, 15.4% crude protein, 0.58% digestible lysine) twice daily (8 a.m. and 4 p.m.): up to 70 kg bodyweight 1.8 kg/day; from 70 to 100 kg 2.2 kg/day; above 100 kg 2.8 kg/day. After insemination the feeding level was lowered to 2.5 kg/day.

Oestrus detection

Oestrus detection was done twice daily in the absence of a boar (at approximately 8 a.m. and 4 p.m., immediately after feeding), starting at an age of approximately 180 days. Gilts were considered to be in oestrus when showing a standing response to the Back Pressure Test (Willemse and Boender, 1966) and/or showing vulval signs (swelling and redness). Gilts that had not been in oestrus at approximately 250 days of age were injected intramuscularly with 400 I.U. pregnant mare serum gonadotrophin and 200 I.U. human chorionic gonadotrophin (PG600^{R}, Intervet B.V., Boxmeer, The Netherlands).

Insemination procedure

All gilts were inseminated during their third oestrus. Gilts detected in oestrus at 8 a.m. were inseminated at 4.30 p.m. the same day. Gilts detected in oestrus at 4 p.m., were inseminated at 4.30 p.m. the next day.

The gilts of both social housing systems were randomly assigned to one of two insemination treatments. These were applied at a location apart from the normal housing, to prevent interaction with the other gilts. Gilts were either only artificially inseminated (with a commercial AI dose from one of six randomly chosen GY boars) with as little stimulation as possible, or likewise artificially inseminated followed by introduction to one of three vasectomized Dutch Landrace boars for a period of 20 min. The interactions during this period were registered by means of real time video-recording.

Experimental treatments

Based on social and insemination conditions, four experimental treatments can be discriminated. Social conditions consisted of either individual housing (group 1) or pairwise housing plus daily contact with a vasectomized boar (group 2). In both groups, insemination procedure implicated artificial insemination, either without (AI-) or with contact with a vasectomized boar (AI+).

Slaughter

At Day 9, 10 or 11 after insemination, gilts were weighed and slaughtered at a commercial slaughtering facility. A blood sample was taken and the reproductive tract was removed after stunning and exsanguination. The uterus and cervix were separated from the ovaries, oviducts and mesometrium. Each uterine horn was flushed twice with 30 ml Dulbecco's PBS from the cervical to the ovarian end to collect the embryos. Embryo recovery rate with this method is 100% at Days 8, 9 and 10 of pregnancy (Van der Lende, et al., 1986). The collected material was kept on ice until further processing (approximately 30 min).

Measurements

Uterus and Ovaries. At the laboratory, the uterine horns were weighed and the length was measured. The corpora lutea were dissected from the ovaries, counted and weighed separately.
Embryos. The size of the embryos was measured using a stereo-microscope (magnification 40 times) with an ocular micrometer. The embryos were frozen (-20°C) in 200 μl distilled water, thawed five times and sonicated (2 times 10 s).

DNA content of embryos was measured fluorometrically (Labarca and Paigen, 1980) using Hoechst compound 33258 (Sigma Chemical Co., USA) at a concentration of 1 μg/ml (detection limit 0.04 μg DNA/embryo). Purified calf thymus DNA (Merck, FRG) was used as a reference. DNA content of embryos from litters with an average largest diameter of less than 2.5 mm was measured with Hoechst compound 33258 at a concentration of 0.1 μg/ml (detection limit 0.009 μg DNA/embryo).

Protein content of embryos was measured according to Bradford (1976), with bovine serum albumin (BSA, Sigma Chemical Co.) as a reference (detection limit 0.44 μg protein/embryo). Protein content of embryos from litters with an average largest diameter of less than 2.5 mm was measured using a more concentrated Bradford reagent (detection limit 0.13 μg protein/embryo).
Progesterone. The blood sample obtained at slaughter was centrifuged (1500 g, 15 min) and the serum was stored at -20°C until further analysis. Serum concentration of progesterone was measured by RIA (Helmond et al., 1980), using a specific rabbit antiserum against 4-pregnene-6ß-ol-3,20-dione-hemisuccinate-BSA. The main cross-reacting steroids were pregnenolone (98.0%), corticosterone (2.7%), 17α-hydroxyprogesterone (1.5%) and 20α-hydroxy-4-pregnen-3-one, cortisol, oestrone, oestradiol-17ß, oestradiol-17α, oestriol, androstenedione, dehydroandrosterone and testosterone (all $<0.2\%$). The antiserum was used in a final dilution of 1:15,000. The sensitivity of the assay was 0.1 ng/ml at the 90% B/B_0 level. The intra-assay coefficient of variation was 7.1% and the inter-assay coefficient of variation was 15.6%.

Statistical analysis

Parameters. Weight at oestrus and growth from first to third oestrus were interpolated from

the weekly weighings. Duration of oestrus was estimated by multiplying the number of times oestrus was detected by 12 (hours). Uterine length and weight were calculated as the sum of the lengths and weights of both uterine horns. Absolute embryonic mortality was calculated as the difference between the number of corpora lutea and the number of embryos. Embryonic mortality rate was calculated as the ratio between absolute embryonic mortality and number of corpora lutea.

Model 1: $Y_{ijk} = \mu + S_i + I_j + SI_{ij} + e_{ijk}$

Y_{ijk} = gilt parameters measured before slaughter
μ = overall mean
S_i = effect of Social condition (i = 1,2)
I_j = effect of Insemination condition (j = 1,2)
SI_{ij} = interaction between Social and Insemination conditions
e_{ijk} = random error

Model 2: $Y_{ijkl} = \mu + S_i + I_j + St_k + SI_{ij} + SSt_{ik} + ISt_{jk} + b_l X_{ijkl} + b_{1ij} X_{ijkl} + e_{ijkl}$

Y_{ijkl} = embryonic, uterine and ovarian parameters, measured after slaughter
μ, S_i, I_j, SI_{ij} and e_{ijkl}, as in Model 1
St_k = Stage of pregnancy (k = 1,3)
SSt_{ik} = interaction between Social condition and Stage of pregnancy
ISt_{jk} = interaction between Insemination condition and Stage of pregnancy
b_l = pooled regression coefficient
b_{1ij} = partial regression coefficients for the interaction between Social and Insemination conditions
X_{ijkl} = covariable age or weight at insemination

Analyses. Analyses of variance were performed using the GLM procedure of the SAS package (SAS, 1985). Main factors included in the models were either Social condition (1 vs 2) and Insemination condition (AI- vs AI+) (Model 1) or Social condition, Insemination condition and Stage of Pregnancy (Day 9, 10 or 11) (Model 2 without covariables). Two- and three-way interactions between the factors were analyzed in the initial full factorial model, but excluded from the model if not significant ($P > 0.05$), except for the interaction between Social and Insemination conditions which was always kept in the model. For the parameters number of

corpora lutea, number of embryos, embryonic mortality rate and absolute embryonic mortality, the covariables age or weight at insemination were included (Model 2). Both the pooled covariable and the partial covariable for the interaction between Social and Insemination conditions were analyzed. Embryonic mortality rate, furthermore, has been analyzed including Onset of third oestrus (morning, afternoon) and Social condition as main factors. Their interaction was taken into account as well. Embryonic development was analyzed using litter average as parameter.

Differences in puberty attainment between social groups were analyzed with χ-square analysis. Data on behaviour during the interaction period between boar and gilt after artificial insemination were analyzed with the Mann-Whitney U test for differences between social conditions. Differences in pregnancy rate between social groups and experimental treatments were analyzed with logistic χ-square, using the CATMOD procedure of the SAS package (SAS, 1985). The same procedure was used to analyze differences in pregnancy rate including Onset of third oestrus (morning, afternoon) and Social condition as main factors and taking their interaction into account as well.

Means are expressed as mean $\pm$ standard deviation.

RESULTS

Puberty attainment

A wide range in age (192 to 259 days) and weight (102 to 156 kg) at spontaneous first oestrus existed, equally divided over the social groups. Fewer gilts reached puberty spontaneously in the individually housed gilts (15 out of 30 compared with 28 out of 36; $\chi^2 = 5.57$, $P < 0.025$). In the individually housed group, 15 gilts were given a PG600 injection to induce oestrus. Three gilts did not respond and three showed only one oestrus. These six gilts were slaughtered at the end of the experiment and appeared to have inactive ovaries. In the pairwise housed group eight gilts were given PG600 and all became cyclic. Age and weight at induced oestrus were similar in both social groups, being 263 ± 10 days and 147 ± 12 kg respectively.

Duration of spontaneous first oestrus was longer in the individually housed gilts (40.8 ± 4.1 vs. 30.0 ± 3.0 hours; $P < 0.05$). Second oestrus tended to be longer in the individually housed gilts (42.5 ± 5.2 vs. 31.3 ± 4.2 hours; $P < 0.10$). Duration of third oestrus did not differ significantly between social groups, nor was it significantly affected by insemination conditions.

Of the 66 gilts initially present, 60 became cyclic and were inseminated at third oestrus.

Insemination conditions

In total, 10 individually housed and 18 pairwise housed gilts were given 20 min boar contact after artificial insemination. During this period, 27 gilts were mated. One individually housed gilt did not show a standing response and consequently was not mated. Three individually housed gilts (30.0%) showed an immediate standing response after introduction of the boar, whereas this was the case in 15 of the pairwise housed gilts (83.3%) ($\chi^2=7.97$, $P<0.005$). Duration of standing response latency differed significantly between social groups ($U^9_{18}=35.5$, $P<0.05$). Total duration of first plus eventual second mating (three in the individually housed gilts and five in the pairwise housed gilts) did not differ between social groups ($U^9_{18}=68$, $P>0.05$), median mating duration for individual and pairwise housed gilts being 326 and 378 seconds, respectively.

Table 1. *Pregnancy rates per experimental treatment[1] and per social group.*

		1/AI-	1/AI+	2/AI-	2/AI+	
per experimental treatment						
pregnant/inseminated	(n)	9/14	8/10	17/18	17/18	$\chi^2=0.2$, $P>0.10$
	(%)	(64.3%)	(80.0%)	(94.4%)	(94.4%)	
per social group						
pregnant/inseminated	(n)	17/24		34/36		$\chi^2=4.46$, $P<0.05$
	(%)	(70.8%)		(94.4%)		

[1] 1/AI- individual housing, artificial insemination
1/AI+ individual housing, artificial insemination and mating by a vasectomized boar
2/AI- pairwise housing, artificial insemination
2/AI+ pairwise housing, artificial insemination and mating by a vasectomized boar

Pregnancy rate

Overall pregnancy rate was 85% (51/60) (Table 1). Pregnancy rate was lower in the individually housed gilts ($\chi^2=4.46$, $P<0.05$). Contact with a vasectomized boar after artificial insemination did not affect pregnancy rate in either of the two social groups.

Inseminated gilts not pregnant at slaughter had a longer oestrus than pregnant gilts (first oestrus $P<0.10$, second and third oestrus, $P<0.05$). At third oestrus, 33 gilts showed a first standing oestrus in the morning and 21 in the afternoon. Neither pregnancy rate nor embryonic mortality rate was significantly affected by this in either of the two social groups. The remaining six gilts (three individually housed and three pairwise housed) did not show a

standing response to the back pressure test, and were inseminated based on external signs of oestrus. Of these, two individually housed gilts and all three pairwise housed gilts became pregnant.

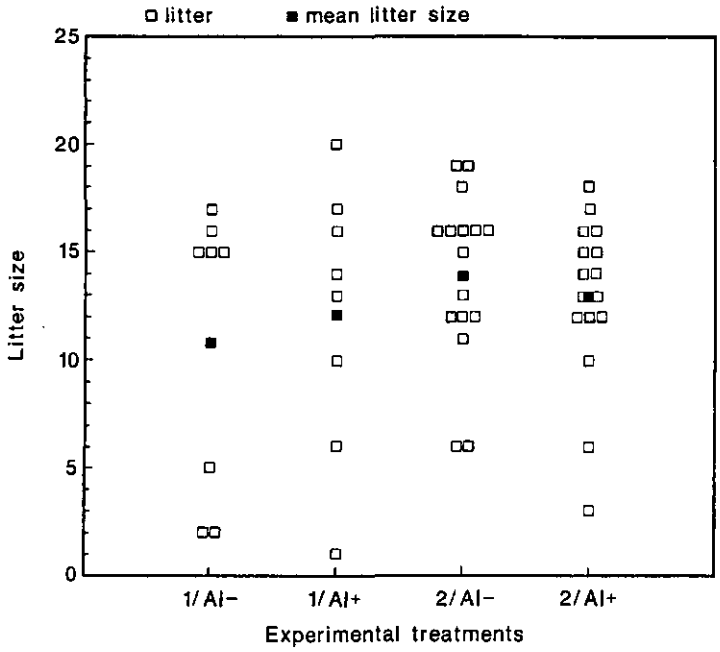

Figure 1. Litter sizes in the experimental treatments.

Embryonic and uterine development

The number of embryos per litter differed considerably (range 1 to 20) but was not significantly different between experimental treatments (Figure 1), overall litter size being 12.8±4.8. Since the number of corpora lutea did not differ between experimental treatments (17.5±3.2), absolute embryonic mortality and embryonic mortality rate were not significantly affected by experimental treatments either.

No significant differences in embryonic development were found between experimental treatments. Average litter diameter, DNA content, and protein content were 3.69±3.20 mm, 2.39±2.96 μg and 57.3±68.7 μg, respectively. Stage of pregnancy significantly affected embryonic development for all three parameters ($P<0.001$), to a same extent in all

experimental treatments.

Development of the uterus did not differ between experimental treatments, average weight and length being 755±149 g and 408±59 cm, respectively. Average progesterone level in blood serum did not differ between experimental treatments either (28.7±6.7 ng/ml).

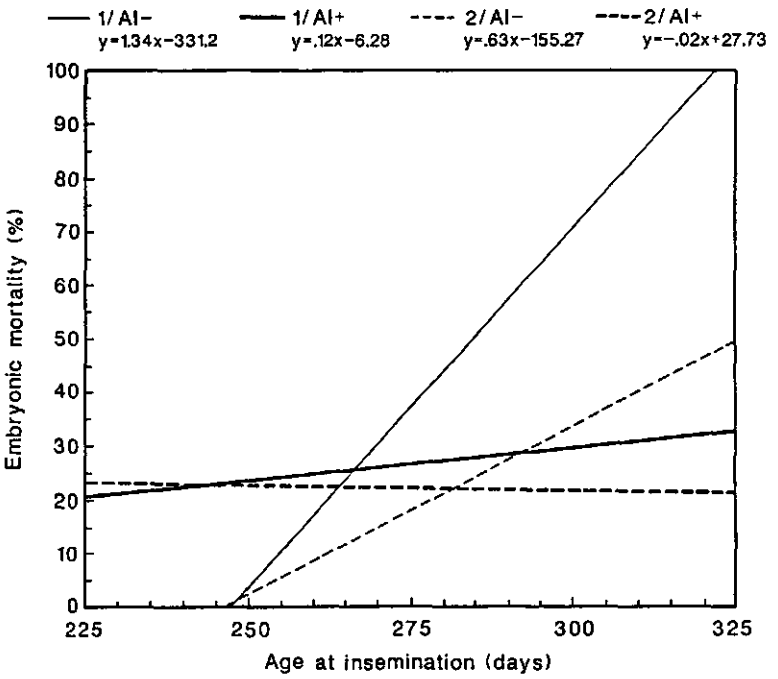

Figure 2. Effect of age at insemination on embryonic mortality rate in the experimental treatments.

Effect of age and weight at insemination on reproductive performance

The number of corpora lutea did not depend on age at insemination, but tended to increase ($P<0.10$) with an increasing weight at insemination (+0.057 CL/kg). The number of embryos, embryonic mortality rate and absolute embryonic mortality were related to age and weight at insemination, the relationships being identical in the two social groups. In the gilts that were only artificially inseminated, fertility was adversely affected by increasing age and weight at insemination. Mating overruled this adverse effect as can be seen in Figure 2 for the relationship between embryonic mortality rate and age at insemination.

The chance of becoming pregnant was affected by weight at insemination, resulting in

different ($P < 0.05$) weights at insemination in pregnant gilts (155.5 ± 15.3 kg) compared to non-pregnant gilts (168.1 ± 11.5 kg).

DISCUSSION

In the present experiment, insemination conditions did not affect pregnancy rate, embryonic mortality rate or average embryonic development around Day 10 after insemination in either of the two groups of gilts differing in social conditions. Mating by a vasectomized male has been shown to have a positive effect on reproductive performance. Mah et al. (1985) found a positive effect on conception rate in pigs as did Restall (1961) in sheep. Pursel et al. (1982) hypothesized that mating might improve fertility by an improved sperm transport, a reduced sperm loss and/or an increased number of sperm reaching the oviduct. The timing and duration of ovulation might be affected too (Signoret et al., 1972; Tilton et al., 1980; Ziecik et al., 1981; Weitze et al., 1990). In the present study, however, both pregnancy rate (individually housed gilts) and embryonic mortality rate (both groups) were susceptible of improvement, but were not significantly affected.

Boar stimuli around insemination have been found to affect farrowing rate and litter size at birth (Hemsworth et al.,1978). Effects of insemination conditions on embryonic mortality rate might, however, only be detectable after Day 11 of pregnancy, since normally, a major part of embryonic mortality occurs after Day 11 of pregnancy (Pope and First, 1985), thereby having a major influence on variation in litter size at birth. On the other hand, in the present experiment embryonic mortality already had reached a high level by Day 11 of pregnancy. It therefore seems unlikely that effects on litter size would have appeared if the gilts in the present experiment would have been allowed to farrow. Also farrowing rate may differ from pregnancy rate around Day 11, since small numbers of embryos seem unable to maintain pregnancy (Polge et al., 1966). The possible return to oestrus of the four gilts with litters of less than five embryos in the present experiment (Figure 1), would, however, not significantly affect the differences in farrowing rate between the insemination conditions in either of the two groups.

Although mating did not affect average embryonic mortality rate, it affected the relationship between age and weight at insemination and embryonic mortality rate identically in both groups. In the non-mated gilts, embryonic mortality rate increased (number of embryos decreased) with age and weight at insemination, whereas in the mated gilts no relationship existed. Apparently, extra stimulation by a boar around insemination is capable of overcoming factors that negatively affect embryonic survival in gilts reaching puberty at a relatively high age. Further research is needed to substantiate this.

Insemination conditions were varied and studied in two groups of gilts in which the

amount of social stimuli differed considerably. The individually housed gilts had no tactile and visual contact with other gilts from an age of approximately 150 days onwards, and the pairwise housed gilts had additional visual and partly tactile contact with gilts in adjacent pens and boar contact once per day from an age of 180 days onwards. Since housing and boar contact were deliberately confounded to create two highly different social environments, differences caused by social treatment can not be attributed to either housing or boar contact. Nonetheless, some interesting effects of social environment have been found that need to be discussed.

Gilts in the two groups showed different behaviour towards the boar after insemination. The pairwise housed gilts showed an almost immediate standing response after boar introduction, whereas in the individually housed gilts a standing response latency existed. These results seem to support the suggestion made by Hemsworth et al. (1978) that individually housed sows experience the boar as a negative stimulus, whereas pairwise housed gilts will more likely experience the boar as a positive stimulus since they are more socially adapted. The boar contact provided in our study in the pairwise housed gilts from 180 days of age onwards presumably even amplified this effect. Duration of mating and standing response during mating, however, were similar in both groups, indicating that once the standing response was elicited, sexual behaviour overruled other behaviour.

In the individually housed group fewer gilts reached puberty spontaneously, fewer gilts responded to oestrus induction with PG600, and pregnancy rate on Day 10 after insemination was lower. The presence or absence of social stimuli have been found to affect puberty attainment, e.g. confinement during rearing has a detrimental effect (Prunier and Etienne, 1984) and it is well known that boar contact can stimulate the attainment of puberty (reviewed by Kirkwood and Hughes, 1982; Dyck, 1988). The absence of social stimuli may cause a chronic stress response (Barnett et al., 1981). The effects of stress on fertility are nearly always adverse and implicate changes in gonadotrophic hormones. Stress may also affect the process and timing of ovulation or even prevent ovulation completely, resulting in lower conception rates and higher embryonic mortality rates (Coubrough, 1985). In the present experiment, pregnancy rates were found to be lower in the individually housed gilts. Embryonic mortality rate was not significantly affected.

Gilts not pregnant at slaughter showed a longer third (and second) oestrus. This may indicate a sub-optimal interval between insemination and ovulation, leading to both lower pregnancy rates and higher embryonic mortality rates (Hunter, 1967; Helmond et al., 1986). A sub-optimal timing of insemination might be expected in a number of gilts, since irrespective of the moment of onset of third oestrus (morning or afternoon), all gilts were inseminated in the first subsequent afternoon. However, no differences in pregnancy rate nor embryonic mortality rate due to this difference in the timing of insemination could be detected.

In conclusion, insemination conditions did not influence reproductive performance

around Day 10 of pregnancy in terms of pregnancy rate, average embryonic mortality rate or average embryonic development. However, insemination conditions affected the relationship between age or weight at insemination and embryonic mortality identically in the two social groups studied. Further study will be needed to substantiate this. Reproductive performance and behaviour of the gilts towards the boar after insemination depended on the social history of the gilts. In contrast to Hemsworth et al. (1978), however, effects of insemination conditions on reproductive performance were independent of the social history of the gilts.

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REFERENCES

Barnett, J.L., Cronin, G.M. and Winfield, C.G., 1981. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. Gen. Comp. Endocrinol., 44: 219-225.

Barnett, J.L., Hemsworth, P.H. and Cronin, G.M., 1982. The efffect of mating on plasma corticosteroids in the female pig and the influence of individual and group penning on this response. Gen. Comp. Endocrinol., 47: 516-521.

Barnett, J.L., Hemsworth, P.H., Winfield, C.G. and Hansen, C., 1986. Effects of social environment on welfare status and sexual behaviour of female pigs. I. Effects of group size. Appl. Anim. Behav. Sci., 16: 249-257.

Booth, W.D., 1984. A note on the significance of boar salivary pheromones to the male effect on puberty attainment in gilts. Anim. Prod., 39: 149-152.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Claus, R., Hoang-Vu, C., Ellendorff, F., Meyer, H.D., Schopper, D. and Weiler, U., 1987. Seminal oestrogens in the boar: origin and functions in the sow. J. Steroid Biochem., 27: 331-335.

Coubrough, R.I., 1985. Stress and fertility. A review. Onderstepoort J. Vet. Res., 52: 153-156.

Döcke, F. and Worch, H., 1963. Untersuchungen über die Uterusmotilität und die Paarungsreaktionen der Sau. Zuchthygiene, 7:169-178.

Dyck, G.W., 1988. Factors influencing sexual maturation, puberty and reproductive performance in the gilt. Can. J. Anim. Sci., 68: 1-3.

Dziuk, P.J., 1987. Embryonic loss in the pig: an enigma. In: J.L. Barnett, E.S. Batterham, G.M. Cronin, C. Hansen, P.H. Hemsworth, D.P. Hennessy, P.E. Hughes, N.E. Johnston and R.H. King (Editors). Manipulating pig production. Australasian Pig Science Association, Werribee, Vic., pp. 28-39.

England, D.C. and Spurr, D.T., 1969. Litter size of swine confined during gestation. J. Anim. Sci., 28: 220-223.

Helmond, F., Simons, P.A. and Hein, P.R., 1980. The effect of progesterone on estrogen-induced luteinizing hormone and follicle-stimulating hormone release in the female rhesus monkey. Endocrinology, 107: 478-485.

Helmond, F., Aarnink, A. and Oudenaarden, C., 1986. Periovulatory hormone profiles in relation to embryonic development and mortality in pigs. In: J.M. Sreenan and M.G. Diskin (Editors). Embryonic mortality in farm animals. Martinus Nijhoff Publishers, Dordrecht, pp. 119-125.

Hemsworth, P.H., Beilharz, R.G. and Brown, W.J., 1978. The importance of the courting behaviour of the boar on the success of natural and artificial matings. Appl. Anim. Ethol., 4: 341-347.

Hemsworth, P.H., Cronin, G.M. and Hansen, C., 1982a. The influence of social restriction during rearing on the sexual behaviour of the gilt. Anim. Prod., 35: 35-40.

Hemsworth, P.H., Salden, N.T.C.J. and Hoogerbrugge, A., 1982b. The influence of the post-weaning social environment on the weaning to mating interval of the sow. Anim. Prod., 35: 41-48.

Hemsworth, P.H., Barnett, J.C., Hansen, C. and Winfield, C.G., 1986. Effects of social environment on welfare status and sexual behaviour of female pigs. II. Effects of space allowance. Appl. Anim. Behav. Sci., 16: 259-267.

Hughes, P.E., Pearce, G.P., and Paterson, A.M., 1990. Mechanisms mediating the stimulatory effects of the boar on gilt reproduction. J. Reprod. Fertil., Suppl. 40:323-341.

Hunter, R.H.F., 1967. The effects of delayed insemination on fertilization and early cleavage in the pig. J. Reprod. Fertil., 13: 133-147.

Kirkwood, R.N. and Hughes, P.E., 1982. Puberty in the gilt. The role of boar stimulation. Pig News Inf., 3: 389-394.

Kirsch, J.D., Tilton, J.E., Ziecik, A., Weigl, R., Schaffter, T. and Williams, G.L., 1985. Effects of various mating stimuli on pituitary release of luteinizing hormone in the gilt. Dom. Anim. Endocrinol., 2: 99-104.

Labarca, C. and Paigen, K., 1980. A simple, rapid and sensitive DNA assay procedure. Anal. Biochem., 102: 344-352.

Mah, J., Tilton, J.E., Williams, G.L., Johnson, J.N. and Mardeello, M.J., 1985. The effect of repeated mating at short intervals on reproductive performance of gilts. J. Anim. Sci., 60: 1052-1054.

Mattioli, M., Galeati, G., Conte, F., and Seren, E., 1986. Effect of 5α-androst-16-en-3-one on oxytocin release in estrous sows. Theriogenology, 25: 399-403.

Paterson, A.M., Pearce, G.P. and Hughes, P.E., 1987. Puberty in gilts handled pleasantly and unpleasantly. In: J.L. Barnett, E.S. Batterham, G.M. Cronin, C. Hansen, P.H. Hemsworth, D.P. Hennessy, P.E. Hughes, N.E. Johnston and R.H. King (Editors). Manipulating Pig Production. Australasian Pig Science Association, Werribee, Vic., pp. 75-76.

Polge, C., Rowson, L.E.A. and Chang, M.C., 1966. The effect of reducing the number of embryos during early stages of gestation on the maintenance of pregnancy in the pig. J. Reprod. Fertil., 12: 395-397.

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

Prunier, A. and Etienne, M., 1984. Effects of confinement on attainment of puberty in gilts. Ann. Rech. Vet., 15: 159-164.

Pursel, V.G., Elliot, D.O. and Newman, C.W., 1982. Mating by vasectomized boars failed to improve fertility in gilts inseminated with frozen semen. Theriogenology, 18: 61-64.

Restall, B.J., 1961. The effect of post-inseminal coitus on percentage of ewes lambing to a single insemination. Austr. Vet. J., 37:70.

SAS, 1985. SAS User's Guide: Statistics, SAS Inst., Inc., Cary, NC, 956 pp.

Signoret, J.P., 1970. Reproductive behaviour of pigs. J. Reprod. Fertil., Suppl. 11: 105-117.

Signoret, J.P., Du Mesnil du Buisson, F. and Mauléon P., 1972. Effect of mating on the onset and duration of ovulation in the sow. J. Reprod. Fertil., 31: 327-330.

Sommer. B., 1980. Sows in individual pens and group housing. Oestrous behaviour, parturition, fertility and damage to limbs. Anim. Breed. Abstr., 48: 619 (Abstract).

Tilton, J.E., Ziecik, A., Kirsch, J.D. and Weigl, R., 1980. Patterns of the LH release in pigs during oestrus. J. Anim. Sci., 51 (Suppl.1): 89-90.

Van der Lende, T., Donker, R. and Hazeleger, W., 1986. Embryonic survival in the pig after insemination with antigen-enriched semen. Theriogenology, 26: 391-396.

Viring, S. and Einarsson, S., 1980a. Influence of boar seminal plasma on the distribution of spermatozoa in the genital tract of gilts. Acta Vet. Scand., 21: 598-606.

Viring, S. and Einarsson, S., 1980b. Effect of boar seminal plasma on uterine and oviductal motility in oestrous gilts. Acta Vet. Scand., 21: 607-616.

Weitze, K.F., Rath, D., Willmen, T., Waberski, D. and Lotz, J., 1990. Advancement of ovulation in the sow related to seminal plasma application before insemination. Reprod. Dom. Anim., 25:61-67.

Willemse, A.H., and Boender, J., 1966. A quantitative and qualitative analysis of oestrus in gilts. Tijdschr. Diergeneesk., 91: 349-363.

Ziecik, A., Tilton, J.E. and Williams, G.L., 1981. Effect of mating on the luteinizing hormone surge in the pig. J. Anim. Sci., 53: 434-438.

Chapter 3

EFFECT OF SOCIAL CONDITIONS ON MATING BEHAVIOUR OF GILTS

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EFFECT OF SOCIAL CONDITIONS DURING REARING ON MATING BEHAVIOUR OF GILTS

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ABSTRACT

The mating behaviour of 28 gilts was studied. The gilts were reared under two different social conditions known to affect both their puberty attainment and reproductive parameters during early pregnancy. The different social conditions were applied from an average age of 137 days onwards. Ten gilts were housed individually, having neither tactile nor visual contact with other pigs. The remaining gilts ($n=18$) were housed pairwise, having additional contact with gilts in adjacent pens and daily boar contact from 180 days of age onwards. At third oestrus, the gilts were artificially inseminated and subsequently introduced to one of three vasectomized boars for a period of 20 min. The gilts were slaughtered 10 ± 1 days after insemination.

The mating behaviour varied considerably between individual gilts, partly because of differences in mating behaviour between the two groups of gilts. More ($P<0.05$) individually housed gilts showed a standing response latency upon introduction of the boar. During this latency period, the individually housed gilts initiated contact with the boar. Once the standing response was elicited, mating behaviour was similar in gilts of both social groups. One individually housed gilt did not show a standing response and consequently was not mated. The mating behaviour of the boars did not differ for the gilts of the two social conditions.

It was concluded that the social conditions of gilts during rearing affected their introductory sexual behaviour. The relationship with reproductive performance during early pregnancy is discussed.

Keywords: social conditions; gilt; mating behaviour.

INTRODUCTION

Boar contact after artificial insemination (AI) has been found to affect the within-litter variation in embryonic development at Day 10 of pregnancy (Soede et al., 1992), consequently possibly affecting embryonic mortality (e.g. Pope and First, 1985). The nature of the effect depended on the social environment of the gilts. In socially isolated gilts, the variation was increased by boar contact after AI, whereas in pairwise housed gilts with daily boar contact, the variation was decreased by boar contact after AI. This interaction between social environment and degree of stimulation at the time of insemination has also been found for litter

size (Hemsworth et al., 1978). These effects might be associated with differences in mating behaviour, as social conditions such as isolated rearing (Hemsworth et al., 1982) and housing conditions such as tethering (England and Spurr, 1969) may affect oestrous and mating behaviour.

Based on the findings described, the present study compares the mating behaviour of the two groups of gilts reared under different social conditions, and the results are discussed in relation to the differences found in reproductive performance between the two groups of gilts.

ANIMALS, MATERIALS AND METHODS

The mating behaviour of 28 Great Yorkshire x British Landrace (GYxBL) gilts (Pig Improvement Company, UK) was studied. The gilts were 127 to 149 (137.4±6.3) days of age upon arrival at the experimental farm. Rearing conditions before arrival are unknown. At arrival, ten gilts were housed in individual pens (3.1 m^2) which did not allow tactile nor visual contact with other gilts. The remaining 18 gilts were housed in pairs (6.2 m^2/pen) and had visual and tactile contact with gilts in adjacent pens. From approximately 180 days of age, one of three vasectomized Dutch Landrace boars was introduced once daily alternately to the pairwise housed gilts (approximately 10 a.m., about 1 minute per pen), to increase social differences between the two groups of gilts. All other environmental conditions were similar in both groups.

Oestrus detection was done twice daily in absence of a boar (at approximately 8 a.m. and 4 p.m., immediately after feeding), starting at an age of approximately 180 days. Gilts were considered to be in oestrus when showing a standing response to the back pressure test (Willemse and Boender, 1966) and/or showing vulval signs (swelling and redness). Gilts that had not been in oestrus at approximately 250 days of age, were injected intramuscularly with 400 I.U. pregnant mare serum gonadotrophin and 200 I.U. human chorionic gonadotrophin (PG600^R, Intervet B.V., Boxmeer, The Netherlands).

All gilts were inseminated during their third oestrus. Gilts detected in oestrus at 8 a.m., were inseminated at 4.30 p.m. on the same day. Gilts detected in oestrus at 4 p.m., were inseminated at 4.30 p.m. the next day. Gilts were artificially inseminated (with a commercial AI dose from one of six randomly chosen GY boars) with as little stimulation as possible. Immediately after artificial insemination, the gilts were, for a period of 20 min, introduced to one of the three vasectomized boars that were also used for daily boar contact in the pairwise housed group. The interactions during this contact period of 20 min were registered by means of real time video recording. The following behaviourial activities initiated by the boar (B) and gilt (G) and their frequencies of occurrence were recorded: ano-genital sniffing (B, G); naso-

nasal contact (B, G); nosing the sides of the gilt (B); mounting (B); aggressive behaviour (G); avoiding behaviour (G). Also recorded were the interval from boar introduction to the first standing response of the gilt and the duration of intromission. The behaviour of the gilts during mating was recorded as 'quiet' (score 1; gilt immobile), 'intermediate' (score 2; gilt partly immobile, partly stepping) or 'restless' (score 3; gilt keeps stepping). The behaviour of the boar and gilts was scored in three periods: the pre-mating period (period before the first mating); the mating period(s); the post-mating period (period after the first mating, excluding the period of an eventual second mating). The data obtained were analyzed with the Mann-Whitney U test for differences between social conditions.

Gilts were slaughtered on Days 9, 10 or 11 after insemination.

RESULTS

Mating behaviour

In Table 1, data on mating behaviour have been summarized for the two groups of gilts. During the 20-min period of contact with a vasectomized boar after artificial insemination, 27 of the 28 gilts were mated; one individually housed gilt did not show a standing response towards the boar, consequently was not mated and was excluded from the analyses.

The number of pre-mating interactions initiated by the boar differed considerably between gilts (1 to 52), but not between the two groups of gilts. Gilt-initiated contact hardly occurred. However, the number of gilts initiating contact before mating differed significantly between the two groups of gilts (Table 1). Furthermore, only three out of nine individually housed gilts showed an immediate standing response after introduction of the boar, whereas in the pairwise housed gilts this was the case for 15 of the 18 gilts ($U^{9}_{18}=35.5$, $P<0.05$).

Within the observation period, a second mating took place in eight out of 27 gilts. The duration of second mating was shorter ($U^{8}_{27}=31$, $P<0.05$) than that of the first. The duration of mating did not differ between social groups.

The three boars did not differ for any of the parameters studied.

Table 1. Behaviour of gilts and vasectomized boars during a period of 20 min after AI. Medians and range of data are presented per group[1]. Significance of differences between groups is tested with the non parametric Mann-Whitney U test.

		individual housing (N=9) median	range	n[2]	pairwise housing (N=18) median	range	n	P	not-pregnant[3] individual	pairwise
Pre-mating										
GILT	initiating contact (n)[4]	1	0 - 7	4/8	0	0 - 0	0/17	P<0.05	2	0
	aggressive behaviour (n)	0	0 - 1	1/8	0	0 - 0			1	0
	avoiding behaviour (n)	0	0 - 5	2/8	0	0 - 0			0	0
	standing response latency (sec)	33	0 - 79	6/9	0	0 - 81	2/17	P<0.05	37	0
BOAR	initiating contact (n)	10	2 - 19		5	1 - 52			19	2
	first mating attempt (sec)	38.5	17 - 83		24	4 - 97			38	16
	mounting attempts unto mating(n)	1	0 - 4	5/8	0	0 - 15	6/17		2	0
Mating										
GILT	restlessness during mating[5]	1	1 - 3		2	1 - 3			2	1
BOAR	time to mating mount(sec)	79	20 - 97		31	4 - 559			80	16
	mating mount to copulation (sec)	45	13 - 75		33	9 - 48			75	40
	duration of first mating (sec)	326	143 - 472		330	110 - 543			240	372
	second mating (n)			3/9			5/18			
	duration of second mating (sec)	149	124 - 156		185	150 - 292			-	-
	total duration of mating (sec)	326	219 - 612		378	110 - 624			240	372
Post-mating										
GILT	initiating contact (n)[4]	7	3 - 33		8	0 - 20	15/17		29	18
	aggressive behaviour (n)	0	0 - 3	1/9	0	0 - 0			3	0
	avoiding behaviour (n)	0	0 - 5	2/9	0	0 - 12	2/17		4	0
BOAR	initiating contact (n)	21	12 - 59		29	11 - 43			59	27

1 One individually housed gilt was excluded since no standing response and therefore no mating occurred

2 The number of gilts in which the behaviourial element occurred divided by the total number of gilts involved, some data are based on 8 (individual housing) respectively 17 (pairwise housing) gilts, because of the poor quality of parts of the two video registrations

3 Behaviour characteristics of the mated, non-pregnant gilts

4 Excluding the aggressive and avoiding behaviour

5 Scaled from 1 (gilt completely immobile) to 3 (restless copulation)

Pregnancy rate

Only three gilts (two out of the ten individually housed and one out of the 18 pairwise housed gilts) were not pregnant at slaughter. One of them was the individually housed gilt that did not show a standing response for the boar; during the 20 minutes, the boar initiated 78 contacts and attempted to mount four times. The gilt initiated naso-nasal and nose-anogenital contacts towards the boar ($n=29$). The aggressive ($n=5$) and avoiding behaviour ($n=18$) of the gilt occurred especially towards the end of the contact period. The other non-pregnant gilts (see Table 1) were mated, their behaviour towards the boar did not differ from that of the pregnant pairwise housed gilts.

The duration of eliciting the standing response by back pressure test was similar for the non-pregnant and pregnant animals, ranging from 24 to 48 hours and 0 to 60 hours, respectively.

DISCUSSION

After introduction of the boar, significantly more individually than pairwise housed gilts showed a latency period until standing response. In this period, contact with the boar was initiated. Furthermore, three individually housed gilts showed aggressive or avoiding behaviour towards the boar before mating. Once the standing response was elicited, mating behaviour was similar in the two groups of gilts. It can be concluded that the two groups of gilts differed in introductory sexual behaviour. Individually housed gilts were not given boar contact during rearing, to increase the differences in social conditions between the two groups of gilts. The differences in mating behaviour may therefore be caused by the lack of social contact or more specifically by the lack of contact with boars. Hemsworth et al. (1982) found that rearing gilts in isolation from boars caused a longer standing response latency and more restless copulations in both individually housed and group housed gilts, suggesting that the lack of specifically boar contact during rearing affected mating behaviour. Signoret (1970) showed that the attraction of oestrous gilts towards a boar was similar for gilts reared in isolation or reared in groups. Therefore, the affected introductory behaviour of gilts reared in isolation in the present experiment seems not to be caused just by the presence of the boar, but primarily by the gilt-directed behaviour of the boar.

In the present experiment, mating behaviour of the gilts and boars and the subsequent chance of pregnancy hardly seem related. This finding, based on only a few non-pregnant gilts, agrees with the finding of Schenk (1967), who did not find a difference in pregnancy rate between gilts that were either restless ($n=54$) or quiet ($n=253$) during mating. Indications exist, however, that mating behaviour of the boar may affect reproductive performance, as Hemsworth et al. (1978) described a strong positive relationship between the number of times

a boar nosed the sides of a sow and subsequent farrowing rate.

The present results on mating behaviour are derived from a larger experiment (a total of 66 gilts were used; 30 individually housed and 36 pairwise housed) in which insemination conditions were varied (artificial insemination vs. artificial insemination followed by 20 min of boar contact). Results showed that variation in embryonic development around Day 10 of pregnancy was increased by boar contact in individually housed gilts and was decreased by boar contact in pairwise housed gilts (Soede et al., 1992), an interaction comparable to that found by Hemsworth et al. (1978) concerning litter size. Therefore, not only mating behaviour but also reproductive processes are affected by the social conditions of the gilts. Furthermore, in the present experiment (66 gilts), individually housed gilts showed a delayed puberty attainment, a decreased response to oestrus induction and a decreased pregnancy rate (Soede et al, 1990). Indications exist that individually housed, chronically stressed gilts (e.g. Barnett et al., 1981; Coubrough, 1985), respond inadequately to acutely stressful situations, such as mating (Barnett et al., 1982). Endocrinological changes associated with stressful conditions especially seem to affect the process of ovulation (Hennessy and Williamson, 1983; Coubrough, 1985). Mating stimuli have also been found to affect the process of ovulation (e.g. Signoret, 1972; Claus, 1990; Weitze et al., 1990). It may be expected that the latter effects depend on the social (stress) conditions of gilts.

It can be concluded that the social conditions of gilts during rearing influence the introductory sexual behaviour of the gilts towards the boar. Reproductive performance was also influenced by the social conditions of the gilts (Soede et al., 1990, 1992). The effect of social conditions of gilts on both mating behaviour and reproductive parameters may be causally related; gilts in a chronic stress situation may react in a physiologically different way to an acutely stressful situation. Studies performed to elucidate this should include study of the timing and duration of ovulation.

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REFERENCES

Barnett, J.L., Cronin, G.M. and Winfield, C.G., 1981. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. Gen. Comp. Endocrinol., 44: 219-225.

Barnett, J.L., Hemsworth, P.H. and Cronin, G.M., 1982. The effect of mating on plasma corticosteroids in the female pig and the influence of individual and group penning on this response. Gen. Comp. Endocrinol., 47: 516-521.

Claus, R., 1990. Physiological role of seminal components in the reproductive tract of the female pig. J. Reprod. Fertil., Suppl. 40: 117-131.

Coubrough, R.I., 1985. Stress and fertility. A review. Onderstepoort J. Vet. Res., 52: 153-156.

England, D.C. and Spurr, D.T., 1969. Litter size of swine confined during gestation. J. Anim. Sci., 28: 220-223.

Hemsworth, P.H., Beilharz, R.G. and Brown, W.J., 1978. The importance of the courting behaviour of the boar on the success of natural and artificial matings. Appl. Anim. Behav. Sci., 4: 341-347.

Hemsworth, P.H., Cronin, G.M. and Hansen, C., 1982. The influence of social restriction during rearing on the sexual behaviour of the gilt. Anim. Prod., 35: 35-40.

Hennessy, D.P. and Williamson, P., 1983. The effects of stress and ACTH administration in hormone profiles, oestrus and ovulation in pigs. Theriogenology, 20: 13-26.

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

Schenk, P.M., 1967. An investigation into the oestrous symptoms and behaviour of sows. Tierzücht. Zücht. Biol., 83: 87-110.

Signoret, J.P., 1970. Reproductive behaviour of pigs. J. Reprod. Fertil., Suppl. 11: 105-117.

Signoret, J.P., du Mesnil du Buisson, F. and Mauléon P., 1972. Effect of mating on the onset and duration of ovulation in the sow. J. Reprod. Fertil., 31: 327-330.

Soede, N.M., Hazeleger, W. and Van der Lende, T., 1990. Effect of insemination conditions on reproductive performance during early pregnancy of gilts kept under different social conditions. Anim. Reprod. Sci., 23: 335-348.

Soede, N.M., Hazeleger, W. and Van der Lende, T., 1992. The influence of insemination conditions on embryonic diversity in gilts depends on their social condition. Submitted to Reprod. Dom. Anim.

Weitze, K.F., Rath, D., Willmen, T., Waberski, D. and Lotz, J., 1990. Advancement of ovulation in the sow related to seminal plasma application before insemination. Reprod. Dom. Anim., 25: 61-67.

Willemse, A.H., and Boender, J., 1966. A quantitative and qualitative analysis of oestrus in gilts. Tijdschr. Diergeneesk., 91: 349-363.

Chapter 4

INFLUENCE OF INSEMINATION CONDITIONS ON EMBRYONIC DIVERSITY DURING EARLY PREGNANCY IN GILTS DEPENDS ON THEIR SOCIAL CONDITION

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THE INFLUENCE OF INSEMINATION CONDITIONS ON EMBRYONIC DIVERSITY IN GILTS DEPENDS ON THEIR SOCIAL CONDITION

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ABSTRACT

A 2x2 factorial design (factors: social environment during rearing and insemination conditions) was applied to gilts. Social conditions differed from an age of approximately 137 days onwards, 30 gilts being individually housed (group 1) and 36 gilts being pairwise housed plus having daily boar contact from approximately 180 days of age onwards (group 2). Insemination conditions of gilts at third oestrus consisted of either only artificially insemination or artificial insemination immediately followed by mating with a vasectomized boar. Gilts were slaughtered at Day 9, 10 or 11 after insemination.

Experimental treatments did not affect average diameter, surface area, volume, DNA- or protein content of litters. Within-litter embryonic diversity (s.d.) increased with average embryonic development in terms of diameter in all four experimental treatments. The rate of increase, however, differed significantly between experimental treatments ($P < 0.05$). Mating by a vasectomized boar resulted in a greater rate of increase in within-litter diversity in individually housed gilts, but a reduced rate of increase in diversity in the pairwise housed gilts. The possible consequences of this effect regarding embryonic mortality after Day 11 of pregnancy are discussed.

Keywords: pig, embryonic diversity, social conditions, insemination conditions.

INTRODUCTION

In the pig, fertilization seems to be an 'all or none' phenomenon (Haines et al., 1959). In the pregnant females, however, an average of about 30% of the fertilized ova are lost during the first five weeks of pregnancy. This loss is referred to as embryonic mortality (recently reviewed by Pope and First, 1985 and Dziuk, 1987). Variation in embryonic mortality between sows is a major cause of differences in litter size at birth (Leymaster et al., 1986).

Accumulating evidence suggests, that within-litter embryonic diversity (measured as diameter), especially around Day 11 of pregnancy, might be a major cause of subsequent embryonic mortality. The more advanced embryos within a litter negatively affect the chance

of survival of the less developed embryos (Pope and First, 1985; Wilmut et al., 1985; Morgan et al., 1987a, b; Pope et al., 1990). Little is known about the factors that influence the degree of diversity in embryonic development within litters. However, duration of ovulation is associated with this diversity (Pope et al., 1988; Xie et al., 1990) and duration and timing of ovulation are influenced by mating stimuli (Signoret et al., 1972; Weitze et al., 1990a, b).

Hemsworth et al. (1972) demonstrated that the effect of mating conditions on reproductive performance (pregnancy rate and litter size) was different in sows under different social conditions (individual vs. pairwise housing). Therefore, in the present experiment, the effect of insemination conditions on embryonic growth and embryonic diversity within litters was studied in two groups of gilts maintained under extremely different social conditions. Embryonic growth and within-litter variation in embryonic development were assessed in terms of diameter, as well as DNA- and protein content.

MATERIALS AND METHODS

Animals

A total of 66 prepuberal crossbred Yorkshire-Landrace gilts (Pig Improvement Company, UK) were used. The gilts were 127 to 149 (137.4±6.3) days of age upon arrival at the experimental farm. Rearing conditions before arrival were unknown.

Experimental Design

At arrival, 30 gilts were housed in individual pens (3.1 m^2) preventing tactile and visual contact with other gilts. The remaining 36 gilts were housed in pairs (6.2 m^2/pen) and had additional visual and tactile contact with gilts in adjacent pens. From approximately 180 days of age, one of three vasectomized boars was introduced once daily to the pairwise housed gilts (approximately 10 a.m., about 1 minute per pen), to increase social differences between the two groups of gilts. Other environmental conditions were similar for both groups.

Gilts were fed a commercial sow ration (12.18 MJ ME per kg, 15.4% crude protein, 0.58% digestible lysine) twice daily (8 a.m. and 4 p.m.): up to 70 kg bodyweight 1.8 kg/day; from 70 to 100 kg 2.2 kg/day; above 100 kg 2.8 kg/day. After insemination the feeding level was reduced to 2.5 kg/day.

Oestrus detection was done in the absence of a boar (at approximately 8 a.m. and 4 p.m., immediately after feeding), starting when gilts were approximately 180 days of age. Gilts were determined to be in oestrus when showing a standing response to the Back Pressure Test (Willemse and Boender, 1966) and/or having swelling and redness of the vulva. Gilts that had not been in oestrus at approximately 250 days of age, were injected intramuscularly with 400 I.U. pregnant mare serum gonadotrophin and 200 I.U. human chorionic gonadotrophin

(PG600^R, Intervet B.V., Boxmeer, The Netherlands). At third oestrus, these gilts were at random equally divided between the insemination conditions.

All gilts were inseminated once at their third oestrus. Gilts detected in oestrus at 8 a.m., were inseminated at 4.30 p.m. the same day. Gilts detected in oestrus at 4 p.m., were inseminated at 4.30 p.m. the next day. The gilts of both social housing systems were assigned randomly to one of two insemination treatments. All gilts were artificially inseminated with minimal stimulation, using a commercial AI dose from one of six randomly chosen GY boars. Half of the gilts of each social housing systems were mated with one of three vasectomized Dutch Landrace boars within a contact period of 20 min immediately following AI. The insemination treatments of all gilts were applied at a location apart from the regular housing to prevent interaction of the boar with other gilts.

On Days 9, 10 or 11 after insemination, gilts were weighed and slaughtered. The reproductive tract was removed immediately after stunning and exsanguination. The uterus and cervix were separated from the ovaries, oviducts and mesometrium. Each uterine horn was flushed twice with 30 ml Dulbecco's PBS (Serva Feinbiochemica gmbh & Co., Heidelberg, FRG) from the cervical to the ovarian end to collect the embryos. Using this method at Days 8, 9 and 10 of pregnancy, all embryos present are flushed from the uterus (Van der Lende et al., 1986). The collected material was kept on ice until further processing (approximately 30 min).

Measurements

At the laboratory, the corpora lutea were dissected from the ovaries and counted. The size of each embryo was measured using a stereo-microscope (magnification of 40x) with an ocular micrometer. The wrinkled embryos were spread out as much as possible. Subsequently, both the largest diameter (di1) and the largest diameter perpendicular to it (di2) were measured. The shape of the embryos was judged as being either spherical or prolate ellipsoid. The embryos were frozen (-20^{o}C) in 200 μl distilled water, thawed five times and sonicated (2 times 10 s).

DNA content of each embryo was measured fluorometrically (Labarca and Paigen, 1980) using Hoechst compound 33258 (Sigma Chemical Co., St. Louis, MO, USA) at a concentration of 1 μg/ml (detection limit 0.04 μg DNA/embryo). Purified calf thymus DNA (Merck. Darmstadt, FRG) was used as the standard. DNA content of embryos from litters for which the average largest diameter was less than 2.5 mm, were measured with Hoechst compound 33258 at a concentration of 0.1 μg/ml (detection limit 0.009 μg DNA/embryo). Protein content of each embryo was measured according to the procedure of Bradford (1976), using Bovine Serum Albumin (BSA, Sigma Chemical Co.) as the standard (detection limit 0.44 μg protein/embryo). Protein content of embryos from litters for which the average largest diameter was less than 2.5 mm were measured using a microassay for protein (detection limit

0.13 μg protein/embryo).

Calculations

Embryonic mortality was calculated as the number of corpora lutea not represented by embryos and expressed as a percentage. The surface area of spherical embryos was calculated as $\pi*(di1)^2$, and their volume as $1/6*\pi*(di1)^3$. The surface area of prolate ellipsoid embryos was calculated as $2*\pi*b^2+[(2*\pi*a*b)/c]*\arcsin c$, in which a = 1/2*di1, b = 1/2 *di2 and $c=\sqrt{(a^2-b^2)}/a$ and their volume as $4/3*\pi*a*b^2$. Diameter analyses were based on the largest diameter (di1) only.

Analyses

Differences in pregnancy rates between treatment groups were analyzed by logistic Chi-square, using the CATMOD procedure of the SAS package (SAS-CATMOD; SAS, 1985).

Litter values for number of corpora lutea, number of embryos, embryonic mortality and embryonic development (diameter (mm), surface area (mm^2), volume (mm^3), DNA content (μg), protein content (μg), DNA content relative to surface area ($\mu g/mm^2$) and relative to volume ($\mu g/mm^3$), protein content relative to surface area ($\mu g/mm^2$) and relative to volume ($\mu g/mm^3$) and protein- relative to DNA content ($\mu g/\mu g$)) have been subjected to analyses of variance (SAS-GLM) according to Model 1. Preliminary analyses had shown that onset of oestrus (a.m. or p.m.) did not affect pregnancy rate, embryonic mortality or embryonic development in any of the treatment groups and therefore was not included in the analyses. In Model 1, none of the interaction effects were found to be significant. Post-hoc analyses of stage of pregnancy effects were performed using the Bonferroni t-test.

Within-litter standard deviations in embryonic development (expressed as diameter, DNA content and protein content) were analyzed according to Model 1 extended with the covariable mean litter development (again expressed as diameter, DNA content and protein content) (the pooled regression coefficient) and the interaction between the experimental treatments (SI_{ij}) and the covariable (the partial regression coefficient). Both the linear and the quadratic component of the covariable were included. In the analyses, one litter containing only one embryo and one litter containing two embryos of which one was ruptured were excluded. None of the interaction effects were found to be significant.

Relationships between average per litter diameter, DNA content and protein content were analyzed using linear regression on ^{e}log-transformed values (SAS-REG). Pearson correlation coefficients were calculated for the pairwise relations between diameter, DNA content and protein content within litters (SAS-CORR).

Model 1: $Y_{ijkl} = \mu + S_i + I_j + St_k + SI_{ij} + SSt_{ik} + ISt_{jk} + e_{ijkl}$

Y_{ijkl} = dependent variable
μ = overall mean
S_i = effect of social condition (i = 1,2)
I_j = effect of insemination condition (j = 1,2)
St_k = day of pregnancy (k = 1,3)
SI_{ij} = interaction between social and insemination conditions
SSt_{ik} = interaction between social condition and day of pregnancy
ISt_{jk} = interaction between insemination condition and day of pregnancy
e_{ijk} = random error

RESULTS

Table 1. Experimental design and descriptive results[1].

	1/AI-	1/AI+	2/AI-	2/AI+	Total
Inseminated (n)	14	10	18	18	60
Pregnant (n)	9 (64%)	8 (80%)	17 (94%)	17 (94%)	51 (85%)
Litters					
Day 9	2	3	6	6	17
Day 10	3	2	6	6	17
Day 11	4 (F)	3	5	5 (F)	17

1 1/AI- individual housing, artificial insemination
1/AI+ individual housing, artificial insemination and mating by vasectomized boar
2/AI- pairwise housing, artificial insemination
2/AI+ pairwise housing, artificial insemination and mating by vasectomized boar
F including one filamentous litter

Reproductive performance

Of the 66 gilts initially present, 60 exhibited oestrus and were inseminated at third oestrus. A total of 51 gilts were pregnant at slaughter (85%) (Table 1). Pregnancy rate was lower ($P<0.05$) for individually housed gilts. At Day 11 of pregnancy two litters had filamentous conceptuses, therefore number of embryos and stage of development of individual embryos in these litters could not be determined because of entanglement of the conceptuses after flushing of the uterus.

Results ($x \pm$s.e.m.) from pregnant gilts indicated that number of corpora lutea (17.4 ± 0.5), number of embryos (12.8 ± 0.7) and embryonic mortality ($25 \pm 4\%$) were not affected by treatment or day of pregnancy. The number of corpora lutea of the non-pregnant animals (17.4 ± 3.5) was not different from that of the pregnant gilts.

Table 2. Effect of stage of pregnancy on embryonic development of individual embryos; protein content, DNA content, diameter, surface, volume and ratios between them. Means of litter means ± s.e.m.[1,2]

	Day 9 (n=17)	Day 10 (n=17)	Day 11 (n=15)[3]
protein content (μg)	4.87 ± 1.50 a	28.98 ± 7.61 a	145.41 ± 12.19 b
DNA content (μg)	.23 ± .06 a	1.06 ± .25 a	6.20 ± .59 b
diameter (mm)	1.15 ± .11 a	2.58 ± .34 a	7.83 ± .57 b
surface (mm^2)	4.96 ± .95 a	27.73 ± 7.56 a	166.42 ± 15.31 b
volume (mm^3)	1.34 ± .36 a	20.13 ± 7.94 a	221.10 ± 28.11 b
protein/DNA	.073 ± .014 a	.053 ± .005 a	.043 ± .002 b
protein/diameter	3.10 ± .66 a	8.57 ± 1.21 a	18.33 ± .81 b
protein/surface	.75 ± .11 a	1.04 ± .07 b	.91 ± .05ab
protein/volume	3.69 ± .47 a	2.67 ± .31 a	.80 ± .09 b
DNA/diameter	.16 ± .02 a	.36 ± .05 a	.79 ± .04 b
DNA/surface	.040 ± .004 a	.047 ± .004 a	.039 ± .003 a
DNA/volume	.207 ± .022 a	.126 ± .016 a	.034 ± .004 b

1 Means with different letters are different ($P<0.05$)
2 Effect of treatment was never significant
3 Results from two litters with filamentous conceptuses were excluded

Embryonic growth

Embryonic development did not differ between treatment groups on any of the days studied. Embryos grew rapidly from Days 9 to 11 of pregnancy, as measured by increases in average litter diameter (seven-fold), DNA content (27-fold, $P<0.05$), protein content (30-fold, $P<0.05$), surface area (34-fold, $P<0.05$) and volume (165-fold, $P<0.05$) (Table 2). Increases in DNA- and protein content relative to diameter were also significant ($P<0.05$). An inconsistent change in the ratio between protein content and surface area of the embryos was also detected ($P<0.05$). The pairwise relationships between the litter averages of the embryonic development parameters diameter (DI), protein content (P) and DNA content (D) were as follows: $P = 2.36*DI^{2.09}$ ($R^2=0.94$), $D = 0.14*DI^{1.88}$ ($R^2=0.94$) and $D = 0.07*P^{.88}$ ($R^2=0.95$). Pairwise correlation coefficients differed considerably between litters, for diameter and DNA content ranging from -0.47 to 0.99, for diameter and protein content ranging from -0.08 to 0.98 and for DNA- and protein content ranging from -0.14 to 0.998. Average within-litter correlations were 0.67, 0.67 and 0.86, respectively.

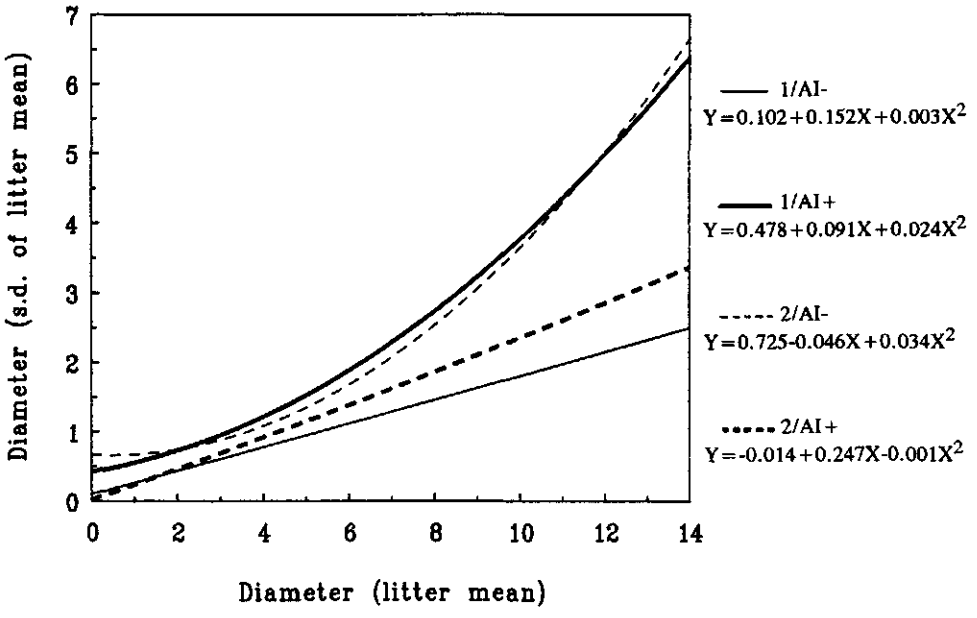

Figure 1. Within-litter standard deviation in embryonic diameter in relation with mean litter diameter for the four experimental groups ($R^2=0.95$, P-interaction <0.05, for legend see Table 1).

Within-litter embryonic diversity

In Model 1, the within-litter standard deviation in diameter, DNA content and protein content were not significantly ($P > 0.05$) affected by the experimental groups (social conditions, insemination conditions or their interaction) or by stage of pregnancy.

However, the within-litter standard deviation in diameter was significantly affected by the mean litter diameter (quadratic component of the pooled regression coefficient; $P < 0.05$); the within-litter diversity in diameter increased as average diameter of all embryos in the litter increased. The rate of increase in diversity with increasing mean litter diameter significantly differed between the experimental groups (quadratic component of the partial regression coefficient; $P < 0.05$); when individually housed gilts were mated by a vasectomized boar there was a greater increase in diversity in embryo diameter compared to the non-mated individually housed gilts. In the pairwise housed gilts, the opposite occurred. In these gilts, mating after artificial insemination reduced the rate of increase in diversity with increasing average embryo diameter (Figure 1).

The within-litter standard deviation in DNA- and protein content were similarly affected by experimental groups, but the differences were not significant ($P > 0.10$).

DISCUSSION

In the present study, social and insemination conditions did not affect embryonic growth, average embryonic development per litter or embryonic mortality. However, within-litter embryonic diversity was significantly affected by an interaction between insemination conditions and social environment of the gilts. Normally, embryonic diversity within litters is large (Perry and Rowlands, 1962; Anderson, 1978; Wright et al., 1983; Stroband et al., 1984; this experiment) and has received much attention, because it appears to influence embryonic mortality (Pope and First, 1985; Wilmut et al., 1985; Morgan et al., 1987a, b). The present study is the first to examine effects of social environment and mating conditions on embryonic development and survival. In the present experiment, mating after artificial insemination increased the rate of increase in diversity in diameter of embryos between Day 9 and 11 of pregnancy in individually housed gilts but reduced the rate of increase in diversity in embryo diameter for pairwise housed gilts. Within-litter embryonic diversity on Day 11 relative to embryonic mortality after Day 11 have only been evaluated by measurements of diameter of embryos (Pope and First, 1985; Pope et al., 1982). In the present experiment, DNA content and protein content of embryos were also considered when comparing diversity in embryonic development, but the differences due to treatment were not significant. Similar changes for the three measures of embryonic development were anticipated because of the high within-litter correlations between the three parameters found in the present experiment. The present results

indicate that within-litter diversity in diameter, DNA content and protein content of embryos are affected similarly by treatments, although effects on diameter are more pronounced.

Positive relationships found between embryonic diversity within litters on Day 11 and subsequent embryonic mortality (reviewed by Pope et al., 1990) together with the results from the present experiment concerning within-litter diversity suggest that embryonic mortality after Day 11 is more likely in individually housed gilts that were mated after artificial insemination and in pairwise housed gilts that were not mated after artificial insemination. Interestingly, Hemsworth et al. (1978) studied litter size of sows under experimental conditions comparable to those in the present experiment, both concerning insemination conditions (courting by a boar prior to artificial insemination vs artificial insemination only) and social conditions (individual vs pairwise housing). If within-litter embryonic diversity at Day 11 actually influences embryonic mortality after Day 11 of pregnancy, differences in litter size between experimental treatments as reported by Hemsworth et al. (1978) are in agreement with results of the present study. They suggested that individually housed sows, in contrast to pairwise housed sows, experience the boar as socially stressing rather than sexually stimulating. Coubrough (1985) stated that effects of stress (negative stimuli) on fertility implicate changes in the profiles of gonadotrophic hormones and are nearly always adverse. Barnett (1982) showed that individually housed gilts, which are chronically stressed (Barnett et al., 1981), respond endocrinologically differently to the acute stress of mating than sows housed in groups of three, and hypothesized that this may subsequently affect reproductive parameters. The present results concerning embryonic diversity seem to confirm this hypothesis. Furthermore, boar stimuli may affect both the timing and duration of ovulation (Signoret et al., 1972; Weitze et al., 1990a, b), and in turn within-litter diversity in embryonic development at Day 11 of pregnancy (Pope et al., 1988). Stress conditions may also negatively affect pregnancy rate (Coubrough, 1985), affecting the timing of ovulation or even obstructing ovulation. This may explain the lower pregnancy rates found in the individually housed gilts in the present experiment, although ovulation was not obstructed in the non-pregnant gilts since they showed a normal ovulation rate. Also reproductive parameters such as puberty attainment and response to oestrus induction with PG600 were negatively affected in the individually housed gilts (Soede et al., 1990).

Results of the present study indicate that within-litter embryonic diversity was influenced by an interaction between insemination conditions and social environment of the gilts. Further research will focus on the causal analyses of the observed phenomena.

REFERENCES

Anderson, L.L., 1978. Growth, protein content and distribution of early pig embryos. Anat. Rec., 190: 143-154.

Barnett, J.L., Cronin, G.M. and Winfield, C.G., 1981. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. Gen. Comp. Endocrinol., 44: 219-225.

Barnett, J.L., Hemsworth, P.H. and Cronin, G.M., 1982. The effect of mating on plasma corticosteroids in the female pig and the influence of individual and group penning on this response. Gen. Comp. Endocrinol., 47: 516-521.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Coubrough, R.I., 1985. Stress and fertility. A review. Onderstepoort J. Vet. Res., 52: 153-156.

Dziuk, P.J., 1987. Embryonic loss in the pig: an enigma. In: J.L. Barnett, E.S. Batterham, G.M. Cronin, C. Hansen, P.H. Hemsworth, D.P. Hennessy, P.E. Hughes, N.E. Johnston & R.H. King (Editors). Manipulating pig production. Australasian pig science association. Werribee, Vic., pp. 28-39.

Haines, C.E., Warnick, A.C. and Wallace, H.D., 1959. The effect of two levels of energy intake on reproductive phenomena in Duroc Jersey Gilts. J. Anim. Sci., 18: 347-354.

Hemsworth, P.H., Beilharz, R.G. and Brown, W.J., 1978. The importance of the courting behaviour of the boar on the success of natural and artificial matings. Appl. Anim. Ethol., 4: 341-347.

Labarca, C. and Paigen, K., 1980. A simple, rapid and sensitive DNA assay procedure. Anal. Biochem., 102: 344-352.

Leymaster, K.A., Christenson, R.K. and Young, L.D., 1986. A biological model to measure uterine potential for litter size in swine. Third World congress on genetics applied to livestock production. XI. Genetics of reproduction, lactation, growth, adaptation, disease, and parasite resistance. Lincoln, Nebraska, pp. 209-214.

Morgan, G.L., Geisert, R.D., Zavy, M.T. and Fazleabas, A.T., 1987a. Development of pig blastocysts in a uterine environment advanced by exogenous oestrogen. J. Reprod. Fertil., 80: 125-131.

Morgan, G.L., Geisert, R.D., Zavy, M.T. and Fazleabas, A.T., 1987b. Development and survival of pig blastocysts after oestrogen administration on Day 9 or Days 9 and 10 of pregnancy. J. Reprod. Fertil., 80: 133-141.

Pope, W.F., Wilde, M.H. and Xie, S., 1988. Effect of electrocautery of nonovulated Day 1 follicles on subsequent morphological variation among Day 11 porcine embryos. Biol. Reprod., 39: 882-887.

Signoret, J.P., du Mesnil du Buisson, F. and Mauléon, P., 1972. Effect of mating on the onset and duration of ovulation in the sow. J.Reprod. Fertil., 31: 327-330.

Perry, J.S. and Rowlands, I.W., 1962. Early pregnancy in the pig. J. Reprod. Fertil., 4: 175-188.

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

Pope, W.F., Maurer, R.R. and Stormshak, F., 1982. Survival of porcine embryos after asynchronous transfer. Proc. Soc. Exp. Biol. Med., 171: 179-183.

Pope, W.F., Xie, S., Broermann, D.M. and Nephew, K.P., 1990. Causes and consequences of early embryonic diversity in pigs. J. Reprod. Fertil., Suppl. 40: 251-260.

SAS. SAS User's guide: Statistics, 1985. SAS Inst., Inc., Cary, NC, 956 pp.

Soede, N.M., Hazeleger, W. and Van der Lende, T., 1990. Effect of insemination conditions on reproductive performance during early pregnancy of gilts kept under different social conditions. Anim.Reprod.Sci., 23: 335-348.

Stroband, H.W.J., Taverne, N. and van de Bogaard, M., 1984. The pig blastocyst: Its ultrastructure and the uptake of protein macromolecules. Cell Tissue Res., 235: 347-356.

Van der Lende, T., Donker, R. and Hazeleger, W., 1986. Embryonic survival in the pig after insemination with antigen enriched semen. Theriogenology, 26: 391-396.

Weitze, K.F., Rabeler, J., Willmen, T. and Waberski, D., 1990a. Interaction between inseminate, uterine and ovarial function in the sow. I. Influence of seminal plasma and oestrogens in the inseminate on intragenital sperm transport, time of ovulation and fertility results in gilts. Reprod. Dom. Anim., 25: 191-196.

Weitze, K.F., Lotz, J.H., Everwand, A., Willmen, T. and Waberski, D., 1990b. Interaction between inseminate, uterine and ovarial function in the sow. II. Investigations into the influencing of ovulation by the use of sperm free media. Reprod. Dom. Anim., 25: 197-204.

Willemse, A.H. and Boender, J., 1966. A quantitative and qualitative analysis of oestrus in gilts. Tijdschr. Diergeneesk., 91: 349-363.

Wilmut, I., Sales, D.I. and Ashworth, C.J., 1985. The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. Theriogenology, 23: 107-119.

Wright, R.W., Grammer, J.C., Bondioli, K., Kuzan, F. and Menino, A., 1983. Protein content and volume of early porcine blastocysts. Anim. Reprod. Sci., 5: 207-212.

Xie, S., Broermann, D.M., Nephew, K.P., Geisert, R.D. and Pope, W.F., 1990. Ovulation and early embryogenesis in swine. Biol. Reprod., 43: 236-240.

Chapter 5

INFLUENCE OF BOAR STIMULATION AFTER INSEMINATION ON EMBRYONIC DIVERSITY AND REPRODUCTIVE PERFORMANCE DURING EARLY PREGNANCY IN INDIVIDUALLY HOUSED GILTS

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ABSTRACT

Effects of insemination conditions (artificial insemination vs artificial insemination followed by 15 min intensive boar contact) on within-litter embryonic diversity and reproductive performance during early pregnancy were studied. Gilts ($n=49$) were individually housed from 162 ± 5 days of age. Of these 49 gilts, 44 gilts became cyclic after PG600 treatment and were inseminated at third oestrus and were slaughtered at Days 5, 6 or 11 after insemination.

In contrast to a previous experiment using similar conditions, reproductive performance in terms of pregnancy rate and embryonic mortality rate was good and boar stimulation did not affect within-litter embryonic diversity. However, boar stimulation increased the number of accessory sperm cells in the zona pellucida ($P<0.05$), showed a tendency to reduce the duration of oestrus ($P=0.09$) and decreased the number of filamentous litters at Day 11 after insemination ($P=0.03$).

It was concluded that boar stimulation after artificial insemination affected reproductive parameters, presumably through an influence on both the transport of sperm and the process of ovulation.

Keywords: pig, embryonic diversity, sperm count, early pregnancy, insemination.

INTRODUCTION

In domestic animals, embryonic mortality is to a large extent responsible for the reproductive losses. In the last decade, accumulating evidence has been presented that, in pigs, within-litter embryonic diversity may be a major cause of embryonic mortality after Day 11 of pregnancy (reviewed by Pope et al., 1990). Variation in embryonic mortality in turn is a major cause of variation in litter size (Leymaster et al., 1986). Factors that influence litter size (e.g. social conditions and insemination conditions, Hemsworth et al., 1978) may, therefore, affect within-litter embryonic diversity. A former experiment showed that, at Day 10 ± 1 after insemination, within-litter embryonic diversity was significantly affected by social environment

during rearing and boar stimulation after artificial insemination (Soede et al., 1992). The present experiment was performed to verify the negative effect of boar stimulation around insemination on within-litter embryonic diversity in individually housed gilts.

MATERIALS AND METHODS

Animals

Great Yorkshire x Dutch Landrace crossbred gilts (n=49) were housed in groups of ten up to 162±5 (149-170) days of age. At that age they were individually housed as described previously (Soede et al., 1990), allowing no visual or tactile contact with other gilts. Oestrus detection was performed twice daily (8 a.m. and 4 p.m.) in absence of a boar, using the back pressure test (Willemse and Boender, 1966) and looking at vulval signs of oestrus. During the first two months of individual housing, none of the gilts showed oestrus. Therefore, all 49 gilts received one dose of PG600^{R} (Intervet B.V., Boxmeer, The Netherlands) to induce oestrus. 24 Gilts did not become cyclic and were given a second PG600 injection. Nine gilts were injected for a third time of which five gilts did not become cyclic. In total, 44 gilts became cyclic.

Treatment

Gilts were inseminated at 4 p.m. of the first day of the second spontaneous oestrus (third oestrus after last PG600-injection). Two insemination treatments were applied. Gilts were either only artificially inseminated (AI with a commercial AI dose of one of six GY-boars) with as little stimulation as possible (AI-), or likewise artificially inseminated followed by contact with one of three vasectomized Dutch Landrace boars for a period of 15 min (AI+).

Embryonic recovery and development

Gilts of both insemination treatments were slaughtered at either Day 5-6 (n=22) or at Day 11 (n=22) after insemination. After slaughter, embryos were flushed from the uterus using 2 x 30 ml Dulbecco's PBS. The Day 5 and Day 6 embryos were subjected to hypotonic treatment (0.6% KCl-solution, 0°C, 10 min) and transferred to a fat free glass slide. Small droplets of methanol/acetic acid (3/1 v/v, 0°C) were added until disruption of the embryo. After drying, xylene treatment (45 min) and staining with 10% Giemsa in PBS-solution (45 min), the number of nuclei and the number of accessory sperm cells from the zona pellucida were counted. Of the Day 11 embryos, diameter was measured and protein content (Bradford, 1976) was assessed as described previously (Soede et al., 1990). Based on diameter, embryos were classified as spherical (<10 mm), ovoid (10-25 mm), tubular (25-100 mm) or

filamentous (> 100 mm).

Statistical Analyses

Analyses of (co)variance were performed using the SAS-package (SAS, 1989). Duration of oestrus was analyzed with O-square and, because of small numbers, frequency of filamentous litters and occurrence of high embryonic mortality rates (>20%) was analyzed with Fisher's exact-P. Mating behaviour, accessory sperm count from the zona pellucida and the percentage of hatched embryos per litter were analyzed with the non-parametric Mann-Whitney U test. Means are expressed as mean±sd unless otherwise indicated.

RESULTS

Oestrus

Of the 49 gilts initially present, 44 became cyclic after PG600 treatment and were inseminated at second spontaneous oestrus. Age at insemination was 300±18 (273 to 334) days and did not differ between treatments. Duration of second spontaneous oestrus differed between treatments; duration of oestrus was less than two days in six of 22 gilts in group AI- compared with 12 of 22 gilts in group AI+ (χ^2= 3.4, P=0.08).

Mating

In the 22 gilts of group AI+, nine gilts were not mated by the boar during the contact period. None of the gilts, either mated or non-mated, showed an immediate standing response upon introduction of the boar. Standing response latency (median (range)) was 46 (16 to 726) s and 66 (21 to 248) s for the mated and non-mated gilts, respectively (P>0.05). Based on subjective criteria, non-mating (n=9) was caused in four cases by the gilt (bad standing response) and in five cases by the boar (low mating activity). In the 13 mated gilts, duration of mating was (median (range)) 137 (66 to 288) s.

Reproductive performance

Pregnancy rate was 100% at Day 5 (n=10) and Day 6 (n=12). At Day 11, pregnancy rate was 91% (10/11) in group AI- and 82% (9/11, one of the non-pregnant gilts suffered from endometritis) in group AI+. Of the AI+ gilts, the mated and non-mated gilts were found to be similar for all parameters concerned.

Table 1. Embryonic recovery (mean±sd) at Day 5-6 and Day 11 in the two treatment groups[1].

		AI-	AI+
		(n=22)	(n=22)
Corpora Lutea (n)		16.3±2.5	15.7±2.8
		(n=11)	(n=11)
Day 5-6	Embryos (n)	15.0±2.7	13.2±2.2
	Embryonic recovery (%)	94.5±5.3	88.4±9.4
		(n=9[2])	(n=9[3])
Day 11	Embryos (n)	14.0±3.5	12.8±3.3
	Embryonic survival (%)	89.7±14.8	79.4±20.7

1 AI- AI with as little stimulation as possible
AI+ AI with as little stimulation as possible, followed by 15 min contact with a vasectomized boar
2 one gilt was not pregnant and in one gilt 100% entangled filamentous embryos were recovered
3 two gilts were not pregnant

Table 1 shows that embryonic recovery at Day 5-6 and embryonic survival at Day 11 were not affected by boar stimulation after artificial insemination (AI- vs AI+, $P>0.05$). At Dat 11, embryonic mortality rate was higher than 20% in five out of ten sows with a duration of oestrus of less than two days compared with zero out of nine sows with a longer oestrus (Fisher's exact-$P=0.02$).

At Day 5-6, the average number of cell cycles per litter and the percentage of hatched embryos per litter (median (range)) were similar for AI- and AI+, being 5.0 ± 1.6 vs 5.8 ± 1.2 ($P>0.05$) and 18 (0 to 100) and 50 (0 to 88), respectively. Boar stimulation significantly ($P<0.02$) increased the median number of accessory sperm cells in the zona pellucida from 22 (10 to 83) to 40 (11 to 196). Figure 1 shows that at Day 11 after insemination, four out of ten gilts in group AI- contained filamentous embryos (100%, 92%, 69% and 44% of the embryos, respectively) compared with zero out of nine gilts in Group AI+ (Fisher's exact-$P=0.03$). For the remaining litters, average diameter ranged between 3.8 and 20.8 mm and average protein content ranged between 34 and 249 μg and were not affected by treatment ($P>0.10$).

Table 2. Embryonic development at Day 11 in the two treatment groups, classified by diameter[1]. Each (I) represents one embryo.

	Spherical 1-2mm	2-6mm	6-10mm	Ovoid 10-25mm	Tubular 25-100mm	Filamentous ≥100mm
Treatment AI-						
1	II	IIIII IIIII IIIII II				
2		IIIII IIII	IIIII II			
3	I	III	IIIII IIIII IIII			
4		III	IIIII I	I		
5	I			IIIII IIII	II	
6				IIIII IIIII II	III	
7		I		II		IIIII IIIII III
8					I	IIIII IIIII II
9					I	IIIII IIIII II
10						100%
Treatment AI+						
1		IIIII IIIII				
2		IIIII IIIII I	IIIII I			
3		III	IIIII IIIII II			
4		II	IIIII	III		
5	I	II	IIIII III	II		
6		I	IIIII IIIII III	III		
7		I	IIII	IIIII		
8		II	IIII	II		
9			II	IIIII IIIII I	I	

1 AI- AI with as little stimulation as possible
AI+ AI with as little stimulation as possible, followed by 15 min contact with a vasectomized boar

Within-litter embryonic diversity

Within-litter embryonic diversity expressed as the within-litter standard deviation of embryonic development was not affected by treatment on either of the days studied (Table 3). At Day 11, gilts with filamentous embryos were excluded from these analyses. At Day 5-6, the within-litter standard deviation of the number of cell cycles was not related with the average number of cell cycles per litter. In contrast, at Day 11, the within-litter standard deviation of both diameter and protein content increased with the respective averages for embryonic development.

Table 3. Relation of within-litter embryonic standard deviation (Y) with average embryonic development (X), excluding (partly) filamentous litters at Day 11.[1,2]

		AI-	AI+	√MSE	R^2
Day 5+6		(n=11)	(n=11)		
Cell cycles (n)	Y (LSM-est.)	0.79	0.64	0.31	0.18
	equation	n.s.	n.s.		
Day 11		(n=6)	(n=9)		
Diameter (mm)	Y (LSM-est.)	4.28	3.49	1.42	0.90
	equation	$Y=-3.01+0.77^{a}X$	$Y=-1.47+0.52^{a}X$		
Protein (μg)	Y (LSM-est.)	47.0	58.6	21.0	0.49
	equation	$Y=-12+0.26^{a}X$	$Y=-15+0.34^{a}X$		

1 LSM-estimates of Y do not differ between treatments ($P>0.05$)

2 AI- AI with as little stimulation as possible
AI+ AI with as little stimulation as possible, followed by 15 min contact with a vasectomized boar

a,a values differ from zero, but do not differ between treatments ($P<0.05$)

DISCUSSION

To study effects of insemination conditions on early reproductive performance and more specifically on within-litter embryonic diversity, gilts were individually housed, allowing no visual or tactile contact with other gilts, from approximately 160 days of age. Furthermore, no boars were present in the housing facility. None of the gilts reached puberty spontaneously before 210 days of age, as was expected under these conditions (Dyck, 1988). Therefore, all gilts were given PG600 to induce oestrus. The gilts showed a high pregnancy rate and a low embryonic mortality rate at third oestrus (second spontaneous oestrus). Generally, individual housing is found to be associated with a decreased reproductive performance (e.g. England and Spurr, 1969; Sommer, 1980; Soede et al., 1990), which is suggested to be due to a chronic stress response (Barnett et al., 1981) interfering with the action of gonadotrophic hormones (Rivier and Rivest, 1991).

Boar stimuli after insemination (15 min boar contact) did not affect within-litter embryonic diversity at Day 5-6, nor at Day 11 after insemination (see Table 2). Based on a previous experiment concerning embryonic diversity at Day 9-11 of pregnancy (Soede et al., 1992) and results of Hemsworth et al. (1978) concerning litter size, in individually housed gilts an increase in diversity was expected. This lack of agreement between experiments (Soede et al., 1992; this experiment) suggests that effects of boar stimulation around insemination on

embryonic diversity may depend on other, at the moment unknown, factors. Early within-litter embryonic diversity seems to be primarily influenced by the duration of ovulation (Pope et al., 1988; Xie et al., 1990). Indications exist that boar stimulation around insemination may influence the process of ovulation (Signoret et al., 1972; Weitze et al., 1990). Therefore, evaluation of effects of boar stimulation on within-litter embryonic diversity should include determination of the duration of ovulation.

Boar stimulation after artificial insemination affected the accessory sperm count (sperm cells bound to the zona pellucida of the embryos). The accessory sperm count is used as an indirect measure for the number of sperm cells present in the oviduct around fertilization. Mating-associated stimuli, such as seminal plasma, volume of semen, the number of sperm cells inseminated have been found to affect the accessory sperm count (e.g. Baker et al., 1968; Rath et al., 1989; Weitze et al., 1990). From the present experiment, it is clear that mating with a vasectomized boar after artificial insemination affected sperm transport. Furthermore, boar stimulation (AI+) tended to increase the number of gilts with a duration of oestrus of less than two days (AI-; 6/22 (27%) vs AI+; 12/22 (55%), $P<0.10$). Since ovulation takes place at a relatively fixed moment in oestrus independent of the length of the oestrous period (Willemse and Boender, 1966; Wagner-Rietschel, 1991), this reduction in the duration of oestrus may be related with the reported advanced ovulation after boar stimulation (Signoret et al., 1972, Weitze et al., 1990). In fact, in some gilts, ovulation may have been advanced to an extent that partial fertilization occurred (Helmond et al., 1986). This would explain the two-fold increase in embryonic mortality rate (n.s.) in AI+-gilts of which especially gilts with a short duration of oestrus had high embryonic mortality rates ($P<0.025$). These results correspond with those of a previous experiment (Soede et al., 1990). In that experiment, in the individually housed gilts, embryonic mortality rate was high and pregnancy rate was low which was combined with a duration of oestrus of less than two days in as many as 17 out of 24 (71%) of the gilts. Boar stimulation after artificial insemination did not influence average embryonic development at Day 5-6, but influenced embryonic development at Day 11 as observed by the decrease in number of advanced ((partly) filamentous) litters ($P<0.05$). Advanced ovulation, as suggested because of the shorter duration of oestrus, together with a reduction in the number of advanced embryos seems contradictory. These seemingly inconsistent effects of boar stimulation on the moment of ovulation can only be clarified when the exact moment of ovulation is known for each individual animal.

In a previous experiment (Soede et al., 1990), low pregnancy rates and high embryonic mortality rates were found at Day 9-11 of pregnancy. In contrast, in the present experiment under similar conditions, reproductive performance in terms of pregnancy rate and embryonic mortality rate was good. One important difference between the two experiments concerns the experimental season; spring and summer (previous) vs autumn and winter (present). It is generally known that reproductive performance is lower during summer (e.g. Claus et al.,

1985). This may have (partly) caused the differences in reproductive performance as measured between the two experiments.

In conclusion, despite similar housing and treatment conditions, results of a previous experiment (Soede et al., 1992) concerning the increase in within-litter diversity after boar stimulation in individually housed gilts could not be confirmed. However, boar stimulation showed a tendency to decrease the duration of oestrus and was found to decrease the number of filamentous litters. Both may have been caused by influences on the process of ovulation. Furthermore, the increased accessory sperm count suggests an effect of boar stimuli on sperm transport. Seemingly similar conditions in two studies resulted in different effects on reproductive performance during early pregnancy (Soede et al., 1990; this experiment) and within-litter embryonic diversity (Soede et al., 1992; this experiment). Determination of the moment and duration of ovulation in individual animals seems an essential tool to clarify the (seemingly) inconsistent results within and between experiments.

REFERENCES

Baker, R.D., Dziuk, P.J. and Norton, H.W., 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. J. Anim. Sci., 27: 88-93.

Barnett, J.L., Cronin, G.M. and Winfield, C.G., 1981. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. Gen. Comp. Endocrinol., 44: 219-225.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Claus, R., Weiler, U. and Hahn, R., 1985. Influence of light and photoperiod on pig prolificacy. J. Reprod. Fertil., Suppl. 33: 185-197.

Dyck, G.W., 1988. Factors influencing sexual maturation, puberty and reproductive efficiency in the gilt. Can. J. Anim. Sci., 68: 1-13.

England, D.C. and Spurr, D.T., 1969. Litter size of swine confined during gestation. J. Anim. Sci., 28: 220-223.

Helmond, F., Aarnink, A. and Oudenaarden. C., 1986. Periovulatory hormone profiles in relation to embryonic development and mortality in pigs. In: J.M. Sreenan and M.G. Diskin (Editors) Embryonic mortality in farm animals. Martinus Nijhoff, Dordrecht, The Netherlands, pp. 119-125.

Hemsworth, P.H., Beilharz, R.G. and Brown, W.J., 1978. The importance of courting behaviour of the boar on the success of natural and artificial matings. Appl. Anim. Ethol., 4: 341-347.

Hennessy, D.P. and Williamson, P., 1983. The effects of stress and ACTH administration in hormone profiles, oestrus and ovulation in pigs. Theriogenology, 20: 13-26.

Leymaster, K.A., Christenson, R.K. and Young, L.D., 1986. A biological model to measure uterine potential for litter size in swine. 3rd World Congr. Gen. Appl. Livest. Prod. pp. 209-214.

Pope, W.F., Wilde, M.H., Xie. S., 1988. Effect of electrocautery of nonovulated Day 1 follicles on subsequent morphological variation among Day 11 porcine embryos. Biol. Reprod., 39: 882-887.

Pope, W.F., Xie, S., Broermann, D.M. and Nephew, K.P., 1990. Causes and consequences of early embryonic diversity in pigs. J. Reprod. Fertil., Suppl. 40: 251-260.

Rath, D., Weitze, K.F., Pena Alfaro, C.E. and Andrade Moura, J.C., 1989. Effects of seminal plasma on the number of accessory sperm cells and fertilization rate in gilts. Zuchthygiene, 24: 123-127.

Rivier, C. and Rivest, S., 1991. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biol. Reprod., 45: 523-532.

SAS, 1990. SAS/STAT User's Guide, Fourth edition, SAS Inst., Cary, NC.

Signoret, J.P., du Mesnil du Buisson, F. and Mauléon, P., 1972. Effect of mating on the onset and duration of ovulation in the sow. J. Reprod. Fertil., 31: 327-330.

Soede, N.M., Hazeleger, W. and Van der Lende, T., 1990. Effect of insemination conditions on reproductive performance during early pregnancy of gilts kept under different social conditions. Anim. Reprod. Sci., 23: 335-348.

Soede, N.M., Hazeleger, W. and Van der Lende, T., 1992. The influence of insemination conditions on variation in early embryonic development in gilts depends on their social condition. Submitted to Reprod. in Dom. Anim.

Sommer, B., 1980. Sows in individual pens and group housing. Oestrous behaviour, parturition, fertility and damage to limbs. Anim. Breed. Abstr., 48: 619.

Wagner-Rietschel, H., 1991. Studies on the estrus and ovulation of sows using transcutaneous sonography. Dissertation Veterinary University, Hannover, 100 pp.

Weitze, K.F., Rabeler, J., Willmen, T. and Waberski, D., 1990. Interaction between inseminate, uterine and ovarial function in the sow. I. Influence of seminal plasma and oestrogens in the inseminate on intragenital sperm transport, time of ovulation and fertility results in gilts. Reprod. Dom. Anim., 25:

191-196.

Willemse, A.H. and Boender, J., 1966. A quantitative and qualitative analysis of oestrus in gilts. Tijdschr. Diergeneesk., 91: 349-363.

Xie, S., Broermann, D.M., Nephew, K.P., Geisert, R.D. and Pope, W.F., 1990. Ovulation and early embryogenesis in swine. Biol. Reprod., 43: 236-240.

Chapter 6

THE DURATION OF OVULATION IN PIGS, STUDIED BY TRANSRECTAL ULTRASONOGRAPHY, IS NOT RELATED TO EARLY EMBRYONIC DIVERSITY

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THE DURATION OF OVULATION IN PIGS, STUDIED BY TRANSRECTAL ULTRASONOGRAPHY, IS NOT RELATED TO EARLY EMBRYONIC DIVERSITY

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ABSTRACT

The duration of ovulation in pigs was studied by transrectal ultrasonography. The number of pre-ovulatory follicles was counted on both ovaries at 30-minute intervals from 36 hours after onset of oestrus (Group A: naturally ovulating sows that were group-housed and were inseminated and caged during scanning) or 40 hours after treatment with human chorionic gonadotrophin (hCG) (Group B: tethered sows that had been induced to ovulate but were not inseminated).

The duration of ovulation was (mean$\pm$sd) 1.8$\pm$0.6 hours (range 0.75 to 3.25) in Group A (n=13) and 4.6$\pm$1.7 hours (range 2.0 to 7.0) in Group B (n=8). The difference was significant ($P<0.01$). In Group A and B sows, respectively, the course of ovulation, expressed as the relation between the relative follicle count (percentage of the maximum follicle count; Y) and the time (percentage of the duration of ovulation; X) was: $Y = 104.3*e^{-0.023*X}$ ($R^2=0.95$) and $Y=98.9*e^{-0.018*X}$ ($R^2=0.92$). The onset of ovulation occurred at approximately two-thirds of the duration of oestrus (Group A: 67$\pm$6%; Group B: 60$\pm$10%).

Group A sows were artificially inseminated and were slaughtered at 98$\pm$8 hours (range 77 to 110) after ovulation. The difference between the maximum follicle count and the corpora lutea count was zero or only one in 81% (21/26) of the ovaries. Embryonic diversity (within-litter s.d. of the number of nuclei or of the number of cell cycles) was not related with the duration of ovulation, neither at the level of ovary nor of sow ($P>0.05$).

In conclusion, transrectal ultrasonography was found to be an appropriate nonsurgical method of studying the duration of ovulation in pigs. The duration of ovulation varied both between sows and between groups of sows and was not related to early embryonic diversity.

Key words: pig, ultrasonography, follicle count, duration of ovulation, embryonic diversity.

INTRODUCTION

A number of experiments, performed during the last decade, provide evidence that within-litter embryonic diversity might be a major cause of embryonic mortality after Day 11 of pregnancy (reviewed by Pope et al., 1990). Although the degree of within-litter diversity differs between sows (Wright et al., 1982), little is known about the factors that influence the

degree of this diversity. There is, however, evidence that the duration of ovulation is associated with the degree of diversity in pre-implantation embryonic development (Pope et al., 1988; Xie et al., 1990a).

A few attempts have been made to determine the duration of ovulation in pigs. Estimates of an average duration in a population sows have been based either upon slaughter of a group of sows (Hunter, 1972; Signoret et al., 1972) or a single follicle count using laparoscopy in a group of sows (Pope et al., 1988), both at the approximate time of ovulation. In individual animals, assessment of the duration of ovulation has been attempted by the use of peritoneal cannulation (Betteridge and Raeside, 1962) or by repeated laparoscopy (Burger, 1952; Brüssow et al., 1990). However, both methods require surgery under general anaesthesia, which may influence the process of ovulation.

The aims of the present study were 1) to evaluate the use of transrectal ultrasonography in assessing the duration of ovulation in individual animals, 2) to establish the duration of ovulation in sows under different conditions and 3) to establish the relation between the duration of ovulation and within-litter embryonic diversity at approximately 100 hours after ovulation.

MATERIAL AND METHODS

Animals, Housing and Feeding

All sows were selected on the basis of internal pelvic diameter allowing for examination per rectum. Group A consisted of ten Great Yorkshire x British Landrace (GYxBL) crossbred sows (Pig Improvement Co., Oxford, UK) and nine Great Yorkshire x Dutch Landrace (GYxDL) crossbred sows (Cofok b.v., Oosterhout, The Netherlands). The sows were housed individually or in pairs in pens of approximately 3x2 m that allowed for visual and partial physical contact with sows in adjacent pens. Group B consisted of 11 GYxBL crossbred sows (Pig Improvement Co., Oxford, UK) that were tethered by neck or breast collar for various periods for at least 2 months prior to assessment of the duration of ovulation, and were intermittently housed like the sows of Group A. All sows were fed a commercial sow ration twice daily (8 a.m. and 4 p.m.).

Hormonal Stimulation of Oestrus

At Day 13, 14 or 15 of the oestrous cycle (onset of oestrus = Day 0), sows were injected twice with 500μg, i.m. of cloprostenol at an 8 to 12 hours interval to induce luteolysis (Estrumate, Coopers Agrovet b.v., Haarlem, The Netherlands). In sows of Group B, 12 hours after the last cloprostenol-injection, 1000 I.U., i.m. of PMSG was administered (Intervet b.v., Boxmeer, The Netherlands). Ovulation was induced with an injection of 750 I.U., i.m. of hCG

72 hours later (Intervet b.v., Boxmeer, The Netherlands).

Oestrus Detection

Oestrus detection was done at eight hour intervals (8 a.m., 4 p.m. and 12 a.m.) with a vasectomized boar. Sows were considered to be in oestrus when they stood to be mounted. The onset of oestrus was defined as moment of first standing response minus four hours. End of oestrus was defined as moment of last standing response plus four hours.

Insemination

Sows in Group A were artificially inseminated every day of oestrus, at approximately 3 p.m. The sows were inseminated with a commercial dosage of mixed-GY spermatozoa.

Ovulation Detection

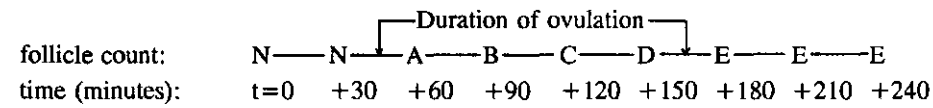
Transrectal ultrasonography was used to study the process of ovulation, as described previously (Soede et al., 1991). An annular array sector scanner (type 150 V, Pie Medical b.v., Maastricht, The Netherlands) with a 5 MHz multiple scan angle transducer was used. To restrict the sows' movements during scanning, the sows were either placed in individual metabolism cages (2.1x0.7 m) after being acclimated (Group A) or tethered by neck or breast collar (Group B).

After the removal of faeces, the transducer was carefully manually brought into the rectum, using abundant boracic ointment as a lubricant. Ovaries were located 35 to 45 cm cranial of the anal sphincter. Their position relative to the transducer in the rectum may vary from lateral to ventral, both within and between animals.

The sows were scanned only once on the first day of standing oestrus. Thereafter, to study the process of ovulation, scanning of both ovaries was performed at 30-minute intervals. In Group A sows, scanning was conducted at different intervals from onset of oestrus (Group A: Attempts 1 to 5). The intervals were dependent on the average of two to three previous oestrus durations for each individual sow. At attempt 6 and onward, scanning was begun at approximately 36 hours after onset of oestrus for all sows (Group A: Attempts 6 to 23). If ovulation had not begun within 20 hours after onset of scanning, sows were given eight hours rest after which scanning was resumed. The sows of Group A in which the duration of ovulation had been missed, were given cloprostenol to induce luteolysis around Day 14 as described and were not scanned for at least one oestrous cycle thereafter. Of the 19 sows, two sows were scanned in two oestrous cycles and one sow was scanned in three oestrous cycles, adding up to 23 attempts to assess the duration of ovulation. Sows in Group B were scanned at 30-minute intervals starting at 32 hours after hCG-treatment (Group B: Attempts 1 to 4) or 40 hours after hCG-treatment (Group B: Attempts 5 to 11). To verify the follicle count after ovulation, sows in Group A and B were scanned once at the day after ovulation.

During the scannings, the number of preovulatory follicles (diameter of antrum ≥4 mm) was counted jointly by two persons. Follicle counts were classified as clear or unclear counts, based on subjective criteria of the two persons counting. The unclear image was generally due to the presence of gas filled intestines or movements of the sows. At irregular intervals, the average diameter of the antrum of one or two follicles on each ovary was determined.

The maximum number of follicles counted on an ovary during the consecutive scan sessions was defined as the maximum follicle count. The onset of ovulation for each ovary was determined based on a consistent decrease in the number of follicles on the ovary and the end of ovulation for each ovary was determined based on the presence of a constant number of a few or no follicles on the ovary at three consecutive scannings. The resulting estimation of the duration of ovulation is shown in the following scheme of successive follicle counts on an ovary (N= follicle count prior to ovulation, A-C= diminishing number of follicles during ovulation, D= follicle count after ovulation):



Slaughter

Sows in Group A of which the duration of ovulation was assessed successfully were slaughtered at 98±8 (77 to 110) hours after onset of ovulation. The number of corpora lutea was counted on both ovaries and the rectum was inspected macroscopically for injuries. To recover the embryos, each oviduct was flushed with 15 ml Dulbecco's PBS (DPBS) from the infundibulum to the uterus. Thereafter, the oviduct was separated from the uterus and each uterine horn was flushed twice with 30 ml DPBS. The embryos were subjected to hypotonic treatment (0.6% KCl-solution, 0°C, 10 min) after which each embryo was placed on a glass slide. Small droplets of methanol/acetic acid (3/1 v/v) were added until disruption of the embryos. After drying, xylene treatment (45 min) and staining with a 10% Giemsa in PBS-solution, the number of nuclei were counted.

Statistical Analyses

Regression analyses, analyses of (co)variance and non-linear regression analyses were performed using the REG, GLM and NLIN procedures, respectively, of the SAS package (SAS, 1990). Evaluation of the transrectal ultrasonography is based on results per ovary for

sows of Group A. Differences between ovaries concerning the number of follicles before ovulation in Group A and in Group B were tested using a t-test for paired samples. Differences between Groups A and B concerning duration of oestrus, onset of ovulation relative to onset of oestrus and duration of ovulation were tested using a t-test for independent samples (Snedecor and Cochran, 1989). Non-linear regression was used to estimate the course of ovulation in Group A and B sows. Embryonic development in sows of Group A was expressed as the number of nuclei and as the number of cell cycles (^{2}log (nuclei count)). Embryonic diversity was expressed as the standard deviation of the nuclei count and of the number of cell cycles, calculated both per ovary (horn) and per litter. Means were expressed as mean$\pm$standard deviation.

RESULTS

Repeatability and Reliability of the Follicle Count (Group A)

The number of follicles counted on an ovary during consecutive scannings was variable, which is illustrated in Figure 1. The total number of scannings before ovulation was 22 ± 11 (range 10 to 40), equivalent to approximately 5 to 20 hours of scanning. The percentage of clear counts of the total counts was $76\pm22\%$ (range 9 to 100), while the percentage of maximum counts of the total counts was $49\pm32\%$ (range 5 to 100). Within the group of sows, a large variation existed in the percentage of clear counts on an ovary and of maximum counts on an ovary. Both the percentage of clear counts (Yc) and the percentage of maximum counts (Ym) on an ovary was negatively related with the maximum follicle count (X): $Yc=120.6-4.97*X$ ($R^2=0.86$, $P=0.002$, sow-effect $P=0.10$) and $Ym=102.7-5.98*X$ ($R^2=0.79$, $P=0.02$, sow-effect $P=0.12$).

Table 1 shows that the maximum pre-ovulatory follicle count, the average number of the clear follicle counts and the average number of the unclear follicle counts predicts the CL count with an accuracy of 0 ± 1 in 81% (21/26), 62% (16/26) and 22% (5/26) of the ovaries, respectively. The difference between the maximum follicle count (before ovulation) and the corpora lutea count (at approximately 100 hours after ovulation) was not significant ($P>0.05$), the difference averaging -0.3 ± 1.5 and -0.2 ± 0.8 for the left and right ovary, respectively. The per sow difference was -0.4 ± 1.8. Regression analyses revealed the following relationship between the maximum follicle count (Y) and the CL count (X) per ovary: $Y=0.61+0.92*X$ ($R^2=0.87$, $P=0.0001$). The deviation between the maximum follicle count and the corpora lutea count did not differ between sows nor was it related with the number of CL ($R^2=0.05$, sow-effect $P=0.73$, CL-effect $P=0.27$).

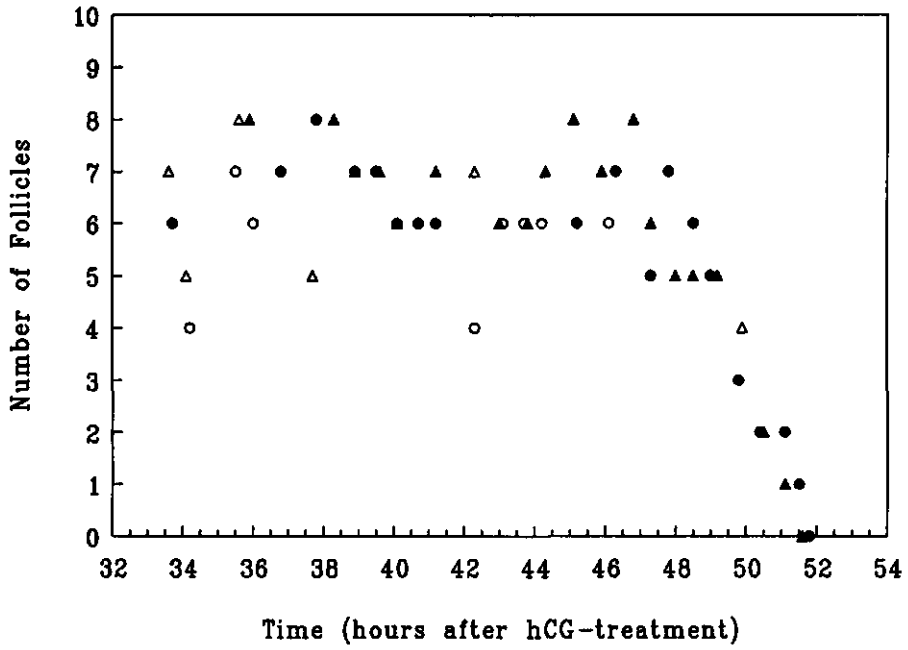


Figure 1. Follicle counts of a sow from Group B, using transrectal ultrasonography from 32 hours after hCG-treatment onwards. The clear counts and the unclear counts are presented on the left (and , respectively) and the right (and , respectively) ovary.

Table 1. Reliability of the follicle counts per ovary based on sows of Group A (n=26).

	Deviation from CL counts			
	0	±1	±2	> ±2
Maximum counts	14	7	4	1
Average of clear counts	6	10	6	4
Average of unclear counts[1]	0	5	8	10

[1] Three ovaries had no unclear counts

Table 2. The average course of ovulation in sows of Group A and B.

		Duration of ovulation (%)[1]				
		0	25	50	75	100
Follicles ovulated (%)[2]						
Group A	(mean±sd)	0	37±14	62±18	87±10	100
	(range)		(17-64)	(38-95)	(56-95)	
Group B	(mean±sd)	0	40±17	58±14	70±17	100
	(range)		(18-75)	(30-75)	(44-92)	

[1] percentage of the total duration of ovulation per sow
[2] percentage follicles ovulated of the maximum follicle count per sow

Moment, Duration and Course of Ovulation

The moment and duration of ovulation was assessed in 13 out of 23 attempts (56%) in Group A. Nine sows ovulated prior to or after the scanning period (two sows ovulated within 24 hours, two sows between 36 and 40 hours, three sows between 44 and 55 hours and two sows between 60 and 68 hours after onset of oestrus), while one sow developed cystic ovaries. In Group B, the moment and duration of ovulation was assessed in eight out of 11 sows (73%). The process of ovulation had started before scanning and two sows developed cystic ovaries.

The maximum follicle count before ovulation in Group A ($n=13$) differed ($P<0.05$) between the left and right ovary (10.8±3.0 and 7.4±2.2, respectively). The maximum follicle count per sow was on average 18.3±3.0 (range 14 to 24). In Group B ($n=8$), the maximum follicle count before ovulation was 10.1±2.4 and 10.3±2.7 for the left and right ovary, respectively ($P>0.05$) and the maximum follicle count per sow was on average 20.3±4.7 (range 15 to 28). The maximum follicle count did not differ between the two groups of sows ($P>0.05$). The diameter of the antrum of the pre-ovulatory follicles varied between 6 and 8 mm and between 5 and 7 mm in Group A and Group B, respectively.

The follicle count after ovulation was zero in 12 of 13 sows in Group A and in one of eight sows in Group B. In the one sow of Group A, two follicles of 3 mm were still present at 11 hours after ovulation. In Group B, an average of 4.0±1.8 (range 2 to 7) follicles of ≤4 mm were present in five of eight sows, and an average of 2.8±2.5 follicles (range 1 to 7) follicles of ≥5 mm remained present in four of eight sows, for at least 11 hours after

ovulation.

The duration of ovulation was on average 1.8±0.6 hours and varied between 0.75 and 3.25 hours in Group A sows (n=13). No significant relationship was found between the duration of ovulation and the maximum follicle count or the number of corpora lutea present, neither per ovary, nor per sow. In Group B sows, the duration of ovulation was 4.6±1.7 hours, varied between 2.0 and 7.0 hours and was not related ($P>0.10$) to the maximum follicle count. The duration of ovulation in Group A sows was significantly shorter ($P<0.01$) than that of Group B sows.

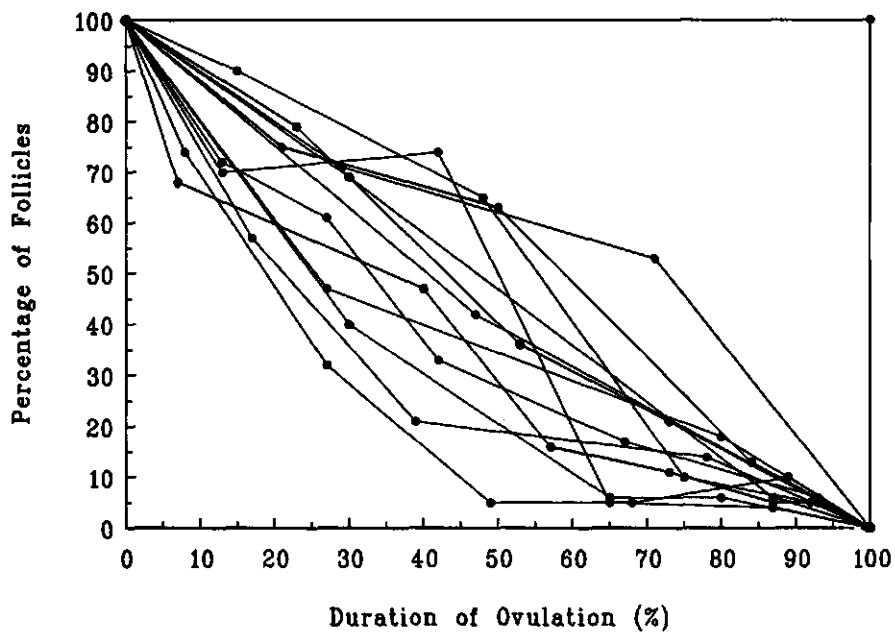


Figure 2. The course of ovulation in sows of Group A (n=13). The duration of ovulation is expressed as percentage of the total duration per sow. The follicle count is expressed as percentage of the maximum follicle count per sow. Each line represents one sow.

The course of ovulation, expressed as the relation between the relative follicle count (percentage of the maximum follicle count; Y) and the time (percentage of the duration of ovulation; X) for Group A was $Y=104.3*e^{-0.023*X}$ ($R^2=0.95$); for Group B it was; $Y=98.9*e^{-0.018*X}$ ($R^2=0.92$). The course of ovulation varied between individuals as can be seen in Table 2 (Groups A and B) and in Figure 2 (Group A). At 50% of the duration of ovulation, the percentage of follicles ovulated varied between 38 and 95% for sows in Group A and between 30 and 75% for sows in Group B (Table 2).

In the sows in which the duration of ovulation was assessed, the onset of ovulation (hours after onset of oestrus) occurred significantly later (47.5 ± 5.7 vs 24.4 ± 13.5; $P<0.01$) and the duration of oestrus was significantly longer (70.8 ± 7.4 vs 39.8 ± 19.3; $P<0.01$) in Group A than in Group B. In Group A ($n=13$), ovulation began at 67 ± 6% (range 58 to 77) of the duration of the oestrus. In Group B ($n=8$), ovulation began at 60 ± 10% (range 47 to 73) of the duration of the oestrus, at 44.5 ± 2.8 (range 40 to 48) hours after hCG-treatment, with one sow ovulating within 40 hours after hCG-treatment. The onset of ovulation as a percentage of the duration of oestrus did not differ significantly between the two groups ($P>0.10$). Furthermore, the relationship between the onset of ovulation (hours after onset of oestrus; Y) and the duration of oestrus (X) did not differ between the two groups of sows and was: $Y=-2.7+0.711*X$ ($R^2=0.82$, group-effect $P=0.35$, $P<0.01$, $n=20$ (excluding one sow in Group B that was not observed in oestrus)).

Ovulation related Embryonic Diversity (Group A)

The corpora lutea count was 19.3 ± 3.3 (range 14 to 25), the number of embryos recovered was 16.1 ± 4.2 (range 12 to 25) and the corresponding embryonic recovery rate was 84 ± 10% (range 71 to 100). Embryonic diversity expressed as the standard deviation of the number of cell cycles per ovary or per sow was not significantly related ($P>0.05$) to the duration of ovulation, age of the embryos or average number of cell cycles. In Figure 3, this lack of relation between the duration of ovulation and embryonic diversity is illustrated. The standard deviation of the number of nuclei (Y) per sow was only significantly related to the age of the embryos (X): $Y=-21.6+0.29*X$ ($R^2=0.57$, $P=0.003$).

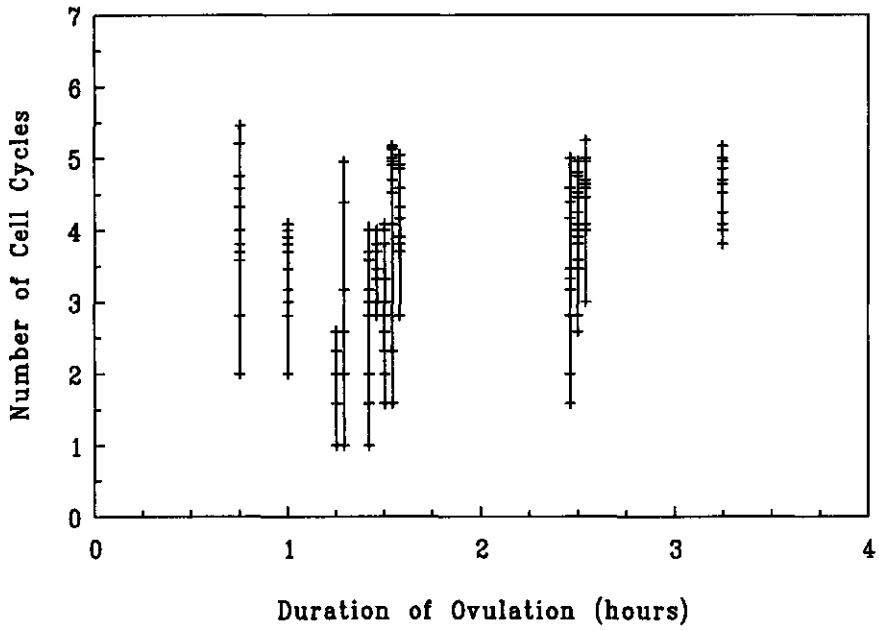


Figure 3. Ovulation related within-litter diversity in embryonic development expressed as the number of cell cycles per embryo. Each vertical bar represents one litter and each (-) represents one embryo.

DISCUSSION

Accurate assessment of the duration of ovulation in individual sows is dependent on repeated accurate counts of the number of follicles on both ovaries, both before and during the process of ovulation. Furthermore, the method of assessing this count should not influence the process of ovulation. The use of laparoscopy (Brüssow et al., 1990) requires surgery under general anaesthesia which may influence the process of ovulation. The use of transcutaneous ultrasonography to study the moment of ovulation in pigs (Weitze et al., 1989) has been found unsuccessful for repeated, accurate counts of follicles (N.M. Soede, unpublished results). Therefore, we used transrectal ultrasonography for assessment of the duration of ovulation in our present study.

Employing transrectal ultrasonography, maximum follicle counts on both ovaries,

counted at 30-minute intervals during 5 to 20 hours before the onset of ovulation, differed only by 0.4 ± 1.8 from the *post mortem* corpora lutea count of 18.6 ± 3.5. On average, therefore, the number of follicles that ovulated was counted accurately. In four of 13 sows (five of 26 ovaries), the difference between the maximum follicle count and the corpora lutea count was more than one. The level of scanning-experience by the operators may affect this.

The number of follicles counted on an ovary during successive scannings before ovulation can vary. In the present experiment, the maximum follicle count per ovary was counted in approximately one of two counts (49%; Group A); this percentage was found to be lower when the total number of follicles present on the ovary was higher ($P < 0.05$). A lower than maximum follicle count may be associated with the configuration of follicles on an ovary e.g. the rate of clustering of follicles. Moreover, unclear counts often result in lower than maximum follicle counts. Unclear counts result from the presence of gas in the intestines or from the movements of sows. The occurrence of unclear counts increases with an increase in the total number of follicles present ($P < 0.05$). The latter might be related to an increased insecurity of the operator when a higher number of follicles is present.

Estimates of the duration of ovulation in oestrus-induced sows vary between 'a few hours' (Pope et al., 1988; Hunter, 1972), <4.5 hours (Signoret et al., 1972), 4 to 5 hours (Kaufman and Holtz, 1982), 4 to 9 hours (Jacob and Elze, 1989) and 6 to >9 hours (Brüssow et al., 1990). In spontaneous ovulating sows the estimates vary between 'a few hours' (Pope et al., 1988), >3.5 hours (Burger, 1952), 3.8 hours (Signoret et al., 1972) and 1 to 7 hours (Jacob and Elze, 1989). No consistent differences between induced and spontaneous ovulating sows have been reported; however, the use of different techniques and breeds between experiments may mask existing differences. In our present experiment, Group A sows ovulated spontaneously and were caged during scanning, whereas Group B sows were induced to ovulate and they were neck- or breast-collared during scanning. Furthermore, Group A sows were inseminated at each day of oestrus. Under these circumstances, the duration of ovulation was found to be significantly shorter for Group A (1.8 ± 0.6 hours) than for Group B (4.6 ± 1.7 hours). First, that sows in Group A were inseminated may have affected the duration of ovulation. Signoret et al. (1972) reported a shorter duration of ovulation in sows that were mated compared to the nonmated controls (0.9 vs 3.8 hours, respectively). Whether or not artificial insemination may have comparable effects on the duration of ovulation is unclear although effects have been found on the timing of ovulation (Weitze et al., 1990a, b). Second, hormonal regulation of follicle growth and ovulation may increase both follicular diversity (Hunter and Wiesak, 1990; Wiesak et al., 1990) and embryonic mortality (reviewed by Van der Lende and Schoenmaker, 1990). Diversity in follicular development and oocyte maturation (Xie et al., 1990b, c) may be related with diversity in timing of ovulation (Pope et al., 1988) which in turn has also been related with an increased embryonic mortality rate (reviewed by Pope et al., 1990). Third, a stress response in sows of Group B due to tethering may have

affected the process of ovulation (Coubrough, 1985).

Using laparotomy, Pope et al. (1988) found that in pigs the first 70% of the follicles rupture nearly simultaneously, with the remaining 30% ovulating during a few hours thereafter. In the present study using transrectal ultrasonography, these results could not be confirmed; in Group A the first 70% of the follicles ruptured at 25 to 85% of the total duration of ovulation, depending on the sow (see Figure 2). These results were similar in Group B.

The within-litter embryonic diversity found in the number of cell cycles at approximately 100 hours after ovulation was quite large (Figure 3) and was not significantly related with the duration of ovulation, which was ≤ 3.25 hours. This result is in contrast to the findings of Xie et al. (1990a). During surgery at 42 hours after hCG-treatment, they recovered and stained the oocytes of nonovulated follicles and returned them to the oviduct. The induced differences in the moment of ovulation within a litter (presumably <3 hours) led to significant differences in within-litter embryonic development at Day 4. Such results, however, should be considered with caution, taking into account the unknown effects of surgery on the process of ovulation and on early embryonic development.

After ovulation, one or more follicles were still present in seven of eight ovulation-induced sows (Group B) and in only one of 13 spontaneous ovulating sows (Group A). At approximately 45 hours after hCG-induced ovulation, Holtz and Schlieper (1990) found higher numbers of nonovulated follicles in prepuberal gilts that were superovulated with a triple dose compared to a single or double dose of PG600 (400 I.U. PMSG + 200 I.U. hCG, Intervet) at 56 hours prior to hCG-treatment. Therefore, hormonal regulation of oestrus and ovulation may cause the development of persistent follicles, especially when using high dosages of gonadotrophic hormones.

In Group A, 9 sows ovulated prior to or after the scanning period, between <24 and <68 hours after onset of oestrus. Retrospective analysis showed that these sows ovulated at approximately two-thirds of the duration of oestrus, which was similar to that of sows for which the duration of ovulation was assessed (Group A: $67\pm6\%$). This timing of the onset of ovulation during the oestrus corresponds well with the reported 70% using 3-time daily transcutaneous ultrasonography (Wagner-Rietschel, 1991). The onset of ovulation after hCG-treatment varied considerably, from less than 40 hours to 48 hours in 11 sows (Group B). This variation may have important consequences in experiments in which a fixed timing of ovulation is essential (e.g. when follicular development or the moment of insemination is studied).

In summary, transrectal ultrasonography allows for repeated assessment of the number of follicles on pig ovaries and, therefore, is an appropriate nonsurgical method to study the duration of ovulation. The duration of ovulation varied both between sows and between groups of sows; it may be worthwhile to study the causes and consequences of this phenomenon. In a group of sows with a relatively short duration of ovulation ($<$ 3.25 hours) no significant

relationship with within-litter embryonic diversity was found. Therefore, causes of embryonic diversity need further investigation.

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REFERENCES

Betteridge, K.J. and Raeside, J.I., 1962. Observation on the ovary by peritoneal cannulation in pigs. Res. Vet. Sci., 3:390-398.

Brüssow, K.P., Ratky, J., Kanitz, W. and Becker, F., 1990. Determination of the duration of ovulation in gilts by means of laparoscopy. Reprod. Dom. Anim., 25: 184-190.

Burger, J.F., 1952. Sex physiology of pigs. Onderstepoort J. Vet. Res., 25 (Suppl.1): 22-131.

Coubrough, R.I., 1985. Stress and fertility. A review. Onderstepoort J. Vet. Res., 52: 153-156.

Holtz, W. and Schlieper, B., 1990. Unsatisfactory results with the transfer of embryos from gilts superovulated with PMSG and hCG, Theriogenology, 35: 1237-1249.

Hunter, R.H.F., 1972. Ovulation in the pig: timing of the response to injection of Human Chorionic Gonadotropin. Res. vet. Sci., 13: 356-361.

Hunter, M.G. and Wiesak, T., 1987. Evidence for and implications of follicular heterogeneity in pigs. J. Reprod. Fertil., Suppl. 40: 163-177.

Jacob, D. and Elze, K., 1989. Ovulationsverlauf, Embryonenentwicklung und Auftreten von Eizelldegenerationen beim Schwein bei spontanem Östrus sowie nach Brunst- und Ovulationssynchronisation. Mh. Vet.-Med., 44: 422-424.

Kaufman von F. and Holtz, W., 1982. Induction of ovulation in gonadotropin treated gilts with synthetic gonadotropin releasing hormone. Theriogenology, 17: 141-157.

Pope, W.F., Wilde, M.H. and Xie, S., 1988. Effect of electrocautery of nonovulated Day 1 follicles on subsequent morphological variation among Day 11 porcine embryos. Biol. Reprod., 39: 882-887.

Pope, W.F., Xie, S., Broermann, D.M. and Nephew, K.P., 1990. Causes and consequences of early embryonic diversity in pigs. J. Reprod. Fertil., Suppl. 40: 251-260.

SAS, 1990. SAS/STAT User's Guide, SAS Institute, Cary, NC.

Signoret, J.P., du Mesnil du Buisson, F. and Mauléon, P., 1972. Effect of mating on the onset and duration of ovulation in the sow. J. Reprod. Fertil., 31: 327-330.

Snedecor, G.W. and Cochran, W.G., 1989. Statistical Methods. Iowa State University Press, Ames, IA.

Soede, N.M., Van der Lende, T., Kemp, B. and De Koning, M., 1991. Assessment of the duration of ovulation in sows using transrectal ultrasonography. ARTA, 2: 128-129.

Van der Lende, T. and Schoenmaker, G.J.W., 1990. The relationship between ovulation rate and litter size before and after Day 35 of pregnancy in gilts and sows: an analysis of published data. Livestock Prod. Sci., 26: 217-229.

Wagner-Rietschel, H., 1991. Studies on oestrus and ovulation of sows using transcutaneous sonography. Dissertation Veterinary University Hannover, Germany, 100 pp.

Weitze, K.F., Habeck, O., Willmen, T. and Rath, D., 1989. Detection of ovulation in the sows using transcutaneous sonography. Reprod. Dom. Anim., 24: 40-42.

Weitze, K.F., Rabeler, J., Willmen, T. and Waberski, D., 1990a. Interaction between inseminate, uterine and ovarial function in the sow. I. Influence of seminal plasma and oestrogens in the inseminate on intragenital sperm transport, time of ovulation and fertility results in gilts. Reprod. Dom. Anim., 25: 191-196.

Weitze, K.F., Lotz, J.H., Everwand, A., Willmen, T. and Waberski, D., 1990b. Interaction between inseminate, uterine and ovarial function in the sow. II. Investigation into the influencing of ovulation by the use of sperm free media. Reprod. Dom. Anim., 25: 197-204.

Wiesak, T., Hunter, M.G. and Foxcroft, G.R., 1990. Differences in follicular morphology, steroidogenesis and oocyte maturation in naturally cyclic and PMSG/hCG-treated prepubertal gilts. J. Reprod. Fertil., 89: 633-641.

Wright, R.W., Grammer, J., Bondioli, K., Kuzan, F. and Menino, A., 1982. Protein content and volume of early porcine blastocysts. Anim. Reprod. Sci., 5: 207-212.

Xie, S., Broermann, D.M., Nephew, K.P., Geisert, R.D. and Pope, W.F., 1990a. Ovulation and early embryogenesis in swine. Biol. Reprod., 43: 236-240.

Xie, S., Broermann, D.M., Nephew, K.P., Bishop, M.D. and Pope, W.F., 1990b. Relationship between oocyte maturation and fertilization on zygotic diversity in swine. J. Anim. Sci., 68: 2027-2033.

Xie, S., Broermann, D.M., Nephew, K.P., Ottobre, J.S., Day, M.L. and Pope, W.F., 1990c. Changes in follicular endocrinology during final maturation of porcine oocytes. Dom. Anim. Endocrinol., 7: 75-82.

Chapter 7

IN SYNCHRONIZED PIGS, THE DURATION OF OVULATION IS NOT AFFECTED BY INSEMINATION, AND IS NOT A DETERMINANT FOR EARLY EMBRYONIC DIVERSITY

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IN SYNCHRONIZED PIGS, THE DURATION OF OVULATION IS NOT AFFECTED BY INSEMINATION AND IS NOT A DETERMINANT FOR EARLY EMBRYONIC DIVERSITY

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ABSTRACT

The duration of ovulation is said to be related with early embryonic diversity, which in turn is related with embryonic mortality. Indications exist, that the duration of ovulation is influenced by insemination conditions. Therefore, the influence of insemination on the duration of ovulation was studied and the relationship between the duration of ovulation and within-litter early embryonic diversity was studied. To determine whether ovulation assessment (transrectal ultrasonography) influenced early embryonic development, control sows were not scanned.

Group housed, multiparous cyclic sows with an exogenously induced (2x500 μg cloprostenol, 1000 I.U. PMSG, 750 I.U. hCG) oestrus were used. Sows showing onset of oestrus between 8 hours before and 24 hours after hCG-treatment were used in this experiment.

Insemination did not influence ($P > 0.05$) the duration of ovulation of sows, which ovulated between 39 hours and 49 hours after hCG-treatment. The duration of ovulation (mean$\pm$sd(range)) was 2.4 ± 0.7 (1.1 to 4.0) in 15 sows which were artificially inseminated at 22 hours and 30 hours after hCG-treatment. In eight non-inseminated sows, the duration of ovulation was 2.3 ± 0.5 (1.5 to 3.3). The duration of ovulation was not related with within-litter embryonic diversity (within-litter s.d. of the number of nuclei or the number of cell cycles) at 114 to 121 hours after ovulation. Ovulation detection by means of transrectal ultrasonography did not influence fertilization rate, accessory sperm count, early embryonic development and early embryonic diversity and therefore appears to be a worthwhile method to study the moment and duration of ovulation.

In conclusion, the duration of ovulation was never longer than 4.0 hours and was not influenced by insemination. No relation was found between the duration of ovulation and within-litter early embryonic diversity. Furthermore, no indications exist that transrectal ultrasonography significantly affects the processes studied. Therefore, differences in the duration of ovulation between sows do not seem to be an important determinant of differences in within-litter embryonic diversity between sows.

Keywords: pig, ultrasonography, duration of ovulation, insemination, embryonic diversity

INTRODUCTION

Within-litter embryonic diversity is of interest because of its positive relationship with within-litter embryonic mortality rate after Day 11 of pregnancy (see review by Pope et al., 1990). Pope et al. (1988) and Xie et al. (1990a) made plausible that the duration of ovulation is a determinant for this within-litter embryonic diversity. Recently, we have developed the use of transrectal ultrasonography to study the moment and duration of ovulation in pigs (Soede et al., 1991; Soede et al., 1992). In these studies, the duration of ovulation varied from 0.75 to 3.25 hours and no relationship with early within-litter embryonic diversity was found. Furthermore, ovulation was found to last significantly longer in tethered sows with an exogenously induced oestrus which were not inseminated compared to individually and pairwise housed sows with a spontaneous ovulation which were inseminated. Exogenous induction of ovulation, insemination treatment, housing conditions or a combination of these factors may have been responsible for this difference in the duration of ovulation (Soede et al., 1992). In other studies, insemination treatment has been found to influence the process of ovulation (Signoret et al., 1972; Weitze et al., 1990a, b, c).

Based on the above, in the present experiment sows of which oestrus and ovulation were exogenously induced, were used to determine 1) the influence of artificial insemination on the duration of ovulation in sows and 2) the relationship between the duration of ovulation and early embryonic diversity. A control group was included to investigate possible effects of transrectal ultrasonography.

MATERIAL AND METHODS

Animals

Multiparous cyclic Great Yorkshire x Dutch Landrace crossbred sows (GYxDL) were housed in groups of four and were fed a commercial sow ration twice daily (8 a.m., 4 p.m.). Oestrus detection was done twice daily (8.30 a.m., 4.30 p.m.) with a vasectomized boar. Sows were considered to be in oestrus when showing a standing response to the boar. At Day 13, 14 or 15 of the oestrous cycle (Onset of oestrus = Day 0), all sows were injected twice at an 8 hours interval with 500 μg, i.m. of cloprostenol (Estrumate, Coopers Agrovet b.v., Haarlem, The Netherlands) to induce luteolysis. 24 Hours after the last cloprostenol-injection, 1000 I.U., i.m. of PMSG (Intervet b.v., Boxmeer, The Netherlands) was administered. Ovulation was induced with an injection of 750 I.U., i.m. of hCG (Intervet b.v., Boxmeer, The Netherlands) 72 hours thereafter.

Treatment groups

Sows showing onset of oestrus between 8 hours before and 24 hours after hCG-treatment were randomly assigned to one of three treatment groups: Sows of the first group (Ovul+) were inseminated at 22 hours and 30 hours after hCG-treatment with a commercial dosage of mixed-GY spermatozoa. Sows of the second group (Ovul--) were not inseminated. In sows of the first and second group (Ovul+ and Ovul--), duration of ovulation was assessed. The sows of the third group (Control) were inseminated as the sows of the Ovul+ group, but duration of ovulation was not assessed.

Assessment of the duration of ovulation

Basically, the procedure of transrectal ultrasonography is as described previously (Soede et al., 1992). An annular array sector scanner (type 150 V, Pie Medical b.v., Maastricht, The Netherlands) with a 5 MHz multiple scan angle transducer was used. To restrict the sows' movements during scanning, the sows were placed in individual cages (2.1×0.7 m^2) and scanned at 24 hours after hCG-treatment to count the number of pre-ovulatory follicles. To study the process of ovulation, scanning of both ovaries was performed at approximately 30 min intervals from 39 hours to 49 hours after hCG-treatment. At 68 to 70 hours after hCG-treatment, they were scanned once more and returned to their housing facilities.

Before scanning, faeces were removed and the transducer was carefully brought into the rectum manually, using abundant boracic ointment as a lubricant. During subsequent scanning, the size and number of pre-ovulatory follicles (diameter of antrum ≥ 4 mm) was determined jointly by two persons. The maximum number of follicles counted on an ovary during the consecutive scan sessions was defined as the maximum follicle count. The total follicle count is the sum of the maximum follicle count on the left and right ovary for each individual sow. Onset of ovulation for each ovary was determined based on a consistent decrease in the number of follicles on the ovary and end of ovulation for each ovary was determined based on the presence of a constant number of few or no follicles on the ovary at three consecutive scannings.

Embryonic recovery and development

Sows of Ovul+ of which the duration of ovulation was assessed successfully and Control sows were slaughtered at 160 ± 0.2 hours after hCG-treatment. The number of corpora lutea was counted on both ovaries. Each oviduct was flushed with 15 ml Dulbecco's PBS (DPBS) from the infundibulum into the uterus. Thereafter, the oviduct was separated from the uterus and each uterine horn was flushed twice with 30 ml DPBS to collect the embryos+oocytes. Recovery rate was determined as the percentage of embryos+oocytes recovered based on the corpora lutea count. The recovered embryos+oocytes were subjected

to hypotonic treatment (0.6% KCl-solution, 0°C, 10 min) and thereafter placed on a fat free glass slide. Small droplets of methanol/acetic acid (3/1 v/v) were added until disruption. After drying and staining with 10% Giemsa in PBS-solution, the number of nuclei and the number of accessory sperm cells from the zona pellucida were counted. A nuclei count of zero or one was considered unfertilized. Fertilization rate was determined based on the total number of recovered embryos+oocytes.

Statistical analyses

Regression analyses, analyses of (co)variance and non-linear regression analyses were performed using the REG, GLM and NLIN procedure of the SAS package, respectively (SAS, 1990). The course of ovulation is represented by the relation between the relative follicle count (Y; percentage of the maximum follicle count) and the relative duration of ovulation (X; percentage of the duration of ovulation). Non-linear regression of the type $Y=a*e^{-b*X}$ was used to estimate the course of ovulation in sows of Ovul+ and Ovul--. Embryonic diversity was expressed as the standard deviation of the nuclei count and of the number of cell cylces ($=^2$log(nuclei count)) and was analyzed in a model with average litter development (nuclei count and cell cycles, respectively) and duration of ovulation as covariables. Differences in accessory sperm count between treatments were analyzed with the non-parametric Mann-Whitney U test. Means are expressed as mean$\pm$standard deviation (range).

RESULTS

Duration of oestrus (Ovul+, Ovul-- and Control)

In the 15 sows of Ovul+ and 8 sows of Ovul-- that ovulated between 39 hours and 49 hours after hCG-treatment, duration of oestrus was on average 62$\pm$18 (36 to 100) and 67$\pm$16 (48 to 88) hours, respectively. Duration of oestrus was 68$\pm$11 (48 to 84) in the 18 slaughtered Control sows. The differences in average duration of oestrus between groups were not significant.

Onset, duration and course of ovulation (Ovul+ and Ovul--)

In total, 34 sows were scanned to assess the moment and duration of ovulation. Of the inseminated sows (Ovul+), six sows ovulated within 39 hours, 15 sows ovulated between 39 hours and 49 hours and two sows ovulated after 49 hours from hCG-treatment. For the non-inseminated sows (Ovul--), the number of sows was one, eight and two, respectively. The distribution did not differ between the two treatment groups ($\chi^2=1.7$, $P=0.43$). Of the sows that ovulated between 39 hours and 49 hours after hCG-treatment, the total follicle count varied considerably, between 13 and 44. In these animals, the duration of ovulation varied

between animals from 1.1 to 4 hours and was not affected by artificial insemination, the duration of ovulation was averaging 2.4±0.7 and 2.3±0.5 for Ovul+ and Ovul-, respectively (Table 1). The duration of ovulation was not influenced by the total follicle count, neither per ovary nor per sow (R^2=0.06, P=0.09 and R^2=0.03, P=0.46, respectively).

Table 1. Assessment of ovulation in artificially inseminated (Ovul+) and non-inseminated sows (Ovul--). Only sows ovulating between 39 hours and 49 hours after hCG-treatment are included.

	Ovul+	Ovul--
Animals (n)	15	8
Total follicle count (n)	22.8±6.2 (13-39)	25.5±4.0 (18-44)
Onset of ovulation (hours after hCG)	42.9±1.7 (39.8-46.3)	42.5±1.5 (40.3-44.8)
Duration of ovulation (hours)	2.4±0.7 (1.1-4.0)	2.3±0.5 (1.5-3.3)

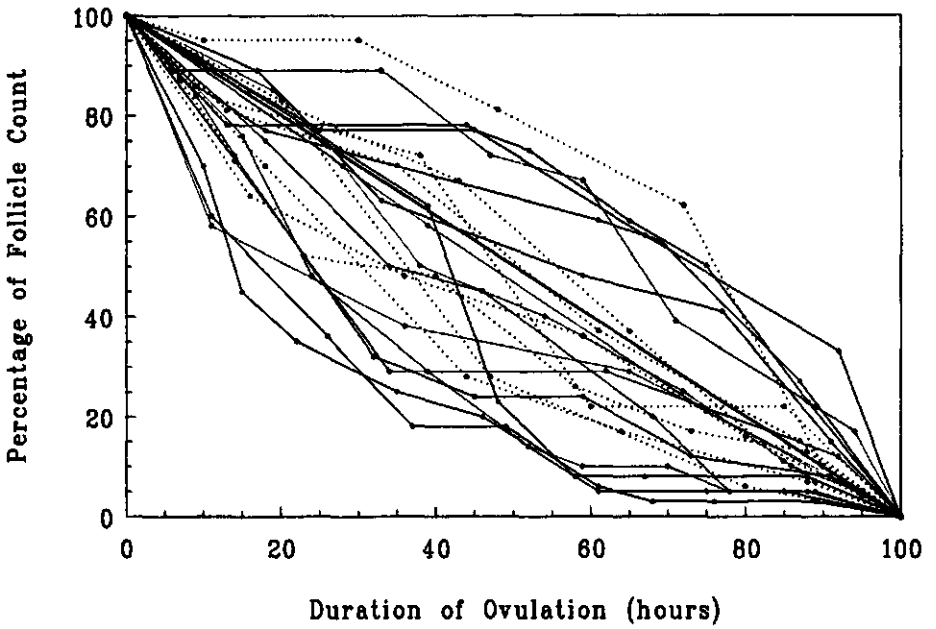

Figure 1. The course of ovulation in sows of Ovul+ (—) and Ovul-- (···). The duration of ovulation is expressed as percentage of the total duration per sow. The follicle count is expressed as percentage of the maximum follicle count per sow. Each line represents one sow.

The course of ovulation, expressed as the relation between the relative follicle count (Y) and the relative duration of ovulation (X) varied between individuals as can be seen in Figure 1. The course of ovulation was not affected by insemination. The course of ovulation was: $Y = 103.4*e^{-0.021*X}$ ($R^2 = 0.91$) for Ovul+, and for Ovul-- it was; $Y = 107.8*e^{-0.020*X}$ ($R^2 = 0.93$).

Follicle and Corpora Lutea counts

In the 30 ovaries, the deviation between the follicle count before ovulation and the corpora lutea count at slaughter was 0, 1, 2 and >2 in 14, 8, 6 and 2 ovaries, respectively. The deviation was on average 0.2 ± 1.5 (-2 to 5) and was not related with the corpora lutea count ($R^2=0.05$, $P=0.25$).

Embryonic development (Ovul+ and Control+)

Sows of Ovul+ that ovulated between 39 hours and 49 hours after hCG-treatment ($n=15$) and Control sows ($n=18$) were slaughtered at 118 ± 2 (114 to 121) hours after ovulation. Total corpora lutea count varied considerably between animals (13 to 44), but was not affected by scanning (Table 2). Of the scanned sows (Ovul+), one sows with 100% fertilization failure was excluded. In the remaining sows, embryonic recovery, fertilization rate and embryonic development were not affected by scanning (Table 2). Embryonic recovery rate was on average 87% and 82% in the two groups, respectively, varying between 45% and 100 % and fertilization rate was on average 91% and 83% in the two groups, respectively, varying between 20% and 100%. Average embryonic development per litter in terms of number of cell cycles varied between 4.7 and 7.4 and did not differ between Ovul+ and Control. Average accessory sperm count of the embryos differed considerably between sows (0.2 to 145). The median of the accessory sperm count was 9 in the scanned sows and 12 in the control sows ($P > 0.05$).

In the control group (Control), two sows with low fertilization rates (20% and 50%, respectively) showed an average embryonic development outside the range of embryonic development in Ovul+ sows (on average 3.9 and 7.4 cell cycles, respectively). Exclusion of these sows resulted in a fertilization rate of 94 ± 12 (63 to 100) and percentage of analyzable embryos of 89 ± 12 (56 to 100) and an average number of cell cycles of 5.8 ± 0.4 (4.8 to 6.4).

Table 2. Embryonic parameters (mean ± sd (range)) in scanned sows (Ovul+) and in control sows (Control).

		Ovul+		Control	
Animals (n)		15		18	
Corpora Lutea (n)		22.6±6.2	(13-39)	24.9±7.0	(17-44)
Embryos (n)					
Recovered (n)		19.6±5.2	(11-32)	20.6±6.8	(9-37)
Recovered (% of C.L.)		87±6	(75-100)	82±13	(45-100)
Fertilized (% of embryos)		91±9	(78-100)[1]	83±22	(20-100)
Analyzable (% of embryos)		88±11	(70-100)[1]	81±22	(20-100)
Embryonic development					
Cell cycles (n)	mean	5.6±0.5	(4.7-6.4)[1]	5.8±0.7	(3.9-7.4)
	sd	0.6±0.3	(0.3-1.3)[1]	0.6±0.4	(0.3-1.7)
Sperm count (n)	median	9	(0.7-39) [1]	12	(0.2-145)

[1] One unfertilized litter was excluded from the analyses

Within-litter embryonic diversity (Ovul+ and Control)

Within-litter embryonic diversity expressed as the standard deviation of the number of cell cycles, ranged from 0.3 to 1.7 and was on average 0.6, both in the scanned sows and in the control sows ($P > 0.05$) (see Table 2). This embryonic diversity (Y_c) tended to be related with the average number of cell cycles per litter (X_c); $Y_c = 1.51 - 0.17*X_c$ ($n=33$, $R^2=0.10$, $P<0.10$). Embryonic diversity after correction for the average number of cell cycles (X_c) was not affected by scanning ($P=0.90$ and $P=0.90$; respectively). Exclusion of the two control sows with on average 3.9 and 7.4 cell cycles somewhat changed the relationship between embryonic diversity and average number of cell cycles $Y_c = 3.00 - 0.42*X_c$ ($n=31$, $R^2=0.33$, $P<0.01$, P-treatment=0.50).

The duration of ovulation was not related with within-litter embryonic diversity expressed as either the standard deviation of the number of cell cycles ($n=15$, P-corrected for $X_c = 0.34$), or the range in number of cell cycles (Figure 2).

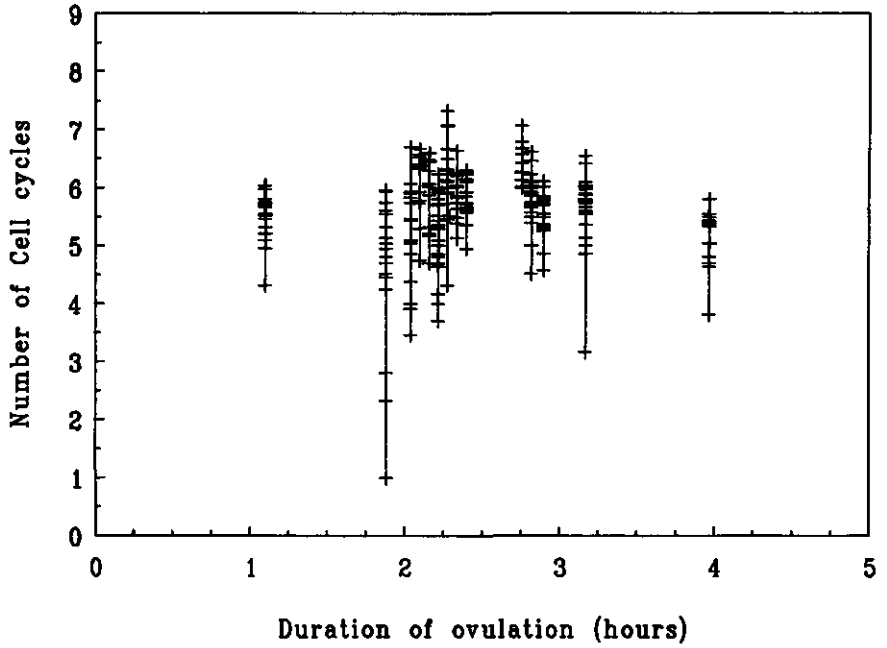

Figure 2. Within-litter embryonic diversity expressed as the number of cell cycles per embryo in relation to the duration of ovulation. Each vertical bar represents one litter and each (+) represents one embryo.

DISCUSSION

In the present experiment, in sows with an exogenously induced oestrus and ovulation, the duration of ovulation varied between 1 and 4 hours and was on average nearly 2.5 hours. The timing and duration of ovulation were not affected by insemination. Furthermore, in the inseminated sows, the duration of ovulation was not related with within-litter embryonic diversity at approximately 120 hours after ovulation.

The timing, duration and course of ovulation did not differ between double inseminated vs non-inseminated sows (see Table 1). In contrast to this, in experiments with spontaneously ovulating sows, insemination conditions have been found to influence both the timing and duration of ovulation. Weitze et al. (1990a, c) showed that, compared to non-inseminated control sows, the timing of ovulation was advanced with 7 hours after insemination with physiological saline and with 14 hours after insemination with sperm plasma compared to non-inseminated control sows. In these experiments, the duration of ovulation was not assessed.

Signoret et al. (1972) used laparoscopy (once per sow) and estimated an average onset and duration of ovulation in a group of sows. They estimated that mating by a boar (twice vs. none) advanced the onset of ovulation with 4 hours and reduced the duration of ovulation from 3.8 to 0.9 hours. Mating by a boar involves more stimuli than insemination and several boar stimuli around insemination have been found to affect the process of ovulation (see review by Soede, 1992). In sows with an exogenously induced oestrus and ovulation, the duration of ovulation was found to be on average 2.3 hours (this experiment) or 4.6 hours (Soede et al., 1992). At this moment it is not clear which environmental factor(s) are primarily responsible for the differences found in the duration of ovulation both within and between experiments.

Early embryonic diversity was assessed at approximately 120 hours after onset of ovulation. At this stage, within-litter embryonic diversity, defined as either the range (Figure 2) or the standard deviation (Table 2) of the number of cell cycles, was not related with the duration of ovulation. This confirms previous results concerning early embryonic diversity at approximately 100 hours after ovulation, using the same procedure (Soede et al., 1992). Based on a series of experiments, it was hypothesized that the duration of ovulation is related with early embryonic diversity which in turn is related with embryonic mortality after Day 11 of pregnancy (reviewed by Pope et al., 1990). Xie et al. (1990a) performed an experiment in which either the first or the last ovulating oocytes were removed from the ovary, were stained and placed in the oviduct. The results showed that, within sows, the first ovulating follicles were the further developed embryos in a litter and the last ovulating follicles were the less developed embryos in a litter, both at approximately 75 hours after ovulation. Our method of transrectal ultrasonography offers an opportunity to relate differences in the duration of ovulation between sows with early embryonic diversity for each individual sow. Using this method, differences between sows in the duration of ovulation (up to 3 hours) were not related with subsequent differences in early embryonic diversity (this experiment; Soede et al., 1992). Therefore, although within litters, differences in the moment of ovulation were found to be related with embryonic development (Xie et al., 1990a), this relationship does not explain differences in within-litter embryonic diversity between litters. Consequently, differences in the duration of ovulation between sows of less than 3 hours may not be a major cause of differences in embryonic mortality after Day 11 of pregnancy.

Since differences in within-litter embryonic diversity between sows are not related with the duration of ovulation, consequently, causes of embryonic diversity lie elsewhere. Shortly before ovulation, diversity in follicle and oocyte development have been reported (Grant et al., 1989; Wiesak et al., 1990; Xie et al., 1990b, c) which have been associated with zygotic development at approximately 10 hours after ovulation (Xie et al., 1990b). Wiesak et al. (1990) showed that the within-litter diversity in oocyte development was increased after

oestrus-induction with PMSG. Furthermore, the rate of early embryonic development may be related with SLA-genotype of the embryos (Ford et al., 1988).

Results concerning the duration of ovulation and subsequent embryonic diversity are only useful when the procedures used do not interfere with the processes involved. Effects of transrectal ultrasonography on the duration of ovulation can not be investigated directly, since no other non-invasive method is available. In the present experiment, non-scanned control sows were used to trace effects of scanning on indirect parameters, such as fertilization rate, embryonic development and embryonic diversity. In the scanned sows (n=34), sows that ovulated too early (within 39 hours from hCG-treatment, n=4) or too late (after 49 hours from hCG-treatment, n=7) were not slaughtered. In two out of the 18 control sows, low fertilization rates (20% and 50%, respectively) were combined with deviating embryonic development (-0.8 and +1.0 cell cycles outside the range of cell cycles in the scanned sows, respectively). Therefore, too late and too early ovulation is assumed in these two control sows. The control group, either including or excluding these two sows, did not differ from the scanned group concerning fertilization rate, accessory sperm count, early embryonic development or within-litter embryonic diversity. Therefore, at 120 hours after ovulation, no effects of repeated transrectal ultrasonography were found on any of the parameters studied. In scanned sows, no relationship was found between the duration of ovulation and within-litter embryonic diversity. Therefore, an effect of transrectal ultrasonography on the process of ovulation can not be completely ruled out.

It is concluded that insemination did not affect the timing, duration or course of an exogenously induced ovulation. Furthermore, the duration of ovulation varied between 1 and 4 hours and was not related with early embryonic diversity.

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REFERENCES

Ford, S.P., Schwartz, N.K., Rothschild, M.F., Conley, A.J. and Warner, C.M., 1988. Influence of SLA-haplotype on preimplantation embryonic cell number in miniature pigs. J. Reprod. Fertil., 84: 99-104.

Grant, S.A., Hunter, M.G. and Foxcroft, G.R., 1989. Morphological and biochemical characteristics during ovarian follicular development in the pig. J. Reprod. Fertil., 86: 171-183.

Pope, W.F., Wilde, M.H. and Xie, S., 1988. Effect of electrocautery of nonovulated Day 1 follicles on subsequent morphological variation among Day 11 porcine embryos. Biol. Reprod., 39: 882-887.

Pope, W.F., Xie, S., Broermann, D.M. and Nephew, K.P., 1990. Causes and consequences of early embryonic diversity in pigs. J. Reprod. Fertil., Suppl. 40: 251-260.

SAS, 1990. SAS/STAT User's guide, Fourth edition, SAS Inst., Cary, NC.

Signoret, J.P., du Mesnil du Buisson, F. and Mauléon, P., 1972. Effect of mating on the onset and duration of ovulation in the sow. J. Reprod. Fertil., 31: 327-330.

Soede, N.M., 1992. Boar stimuli around insemination affect reproductive processes in pigs. A review. Submitted to Anim. Reprod. Sci.

Soede, N.M., Van der Lende, T., Kemp, B. and De Koning, M., 1991. Assessment of the duration of ovulation in sows using ultrasonography. ARTA, 2: 128-129.

Soede, N.M., Noordhuizen, J.P.T.M. and Kemp, B., 1992. The duration of ovulation in pigs, studied by transrectal ultrasonography, is not related with early embryonic diversity. Accepted for publication by Theriogenology.

Wiesak, T., Hunter, M.G. and Foxcroft, G.R., 1990. Differences in follicular morphology, steroidogenesis and oocyte maturation in naturally cyclic and PMSG/hCG-treated prepubertal gilts. J. Reprod. Fertil., 89: 633-641.

Weitze, K.F., Rath, D., Willmen, T., Waberski, D. and Lotz, J., 1990a. Advancement of ovulation in the sow related to seminal plasma application before insemination. Reprod. Dom. Anim., 25: 61-67.

Weitze, K.F., Rabeler, J., Willmen, T. and Waberski, D., 1990b. Interaction between inseminate, uterine and ovarial function in the sow. I. Influence of seminal plasma and oestrogens in the inseminate on intragenital sperm transport, time of ovulation and fertility results in gilts. Reprod. Dom. Anim., 25: 191-196.

Weitze, K.F., Lotz, J., Everwand, A., Willmen, T. and Waberski, D., 1990c. Interaction between inseminate, uterine and ovarial function in the sow. II. Investigations into the influencing of ovulation by the use of sperm free media. Reprod. Dom. Anim., 25: 197-204.

Xie, S., Broermann, D.M., Nephew, K.P., Geisert, R.D. and Pope, W.F., 1990a. Ovulation and early embryogenesis in swine. Biol. Reprod., 43: 236-240.

Xie, S., Broermann, D.M., Nephew, K.P., Bishop, M.D. and Pope, W.F., 1990b. Relationship between oocyte maturation and fertilization on zygotic diversity in swine. J. Anim. Sci., 68: 2027-2033.

Xie, S., Broermann, D.M., Nephew, K.P., Ottobre, J.S., Day, M.L. and Pope, W.F., 1990c. Changes in follicular endocrinology during final maturation of porcine oocytes. Dom. Anim. Endocrinol., 7: 75-82.

GENERAL DISCUSSION

GENERAL DISCUSSION

1. INTRODUCTION

The role of the male in reproduction has not always been recognized. Johnston (1963) described the history of human infertility and remarked that some primitive people ..... " believed that children were the result of some food the mother had eaten. The Euduna tribe insisted that the women bore half-caste children, because their native mothers had partaken the white bread the white settlers had introduced, instead of eating the dark native bread." Adoption of the role of the male semen in mammalian reproduction initially resulted in an overestimate of this role. In ancient Greece (around 400 BC) it was suggested that only the male parent contributes to the genetic character of the offspring, the contribution of the mother being merely nutritional (from: Medvei, 1982). Much has changed since those days. A gradually better understanding of reproductive processes, including discoveries such as that of the sex-chromosomes in 1902 demonstrated the relevance of both female and male in the reproduction of a species. However, the precise role of the male in reproduction has not been completely unravelled, in neither human- nor animal fertility.

In pig husbandry, different insemination conditions such as the application of natural mating versus artificial insemination are considered to result in a similar reproductive performance expressed as farrowing rate and litter size. This success of artificial insemination in recent years suggests that the male contribution to reproduction is nothing but its sperm cells. However, possible differences in reproductive performance after artificial insemination or natural mating have hardly been evaluated experimentally (Crabo, 1990). From studies concerning effects of boar stimulation on reproductive processes under experimental conditions, accumulating evidence has been presented that a whole range of stimuli emitted by the boar contribute to the success of mating. Under field conditions, boars and sows differ amongst others in genetic background, ontogeny and later experience. These differences are known to influence the physiology and behaviour of animals (Levine et al., 1989). Effects of boar stimulation on reproductive performance, expressed as litter size and farrowing rate, may, for example be either positive or negative, depending on the social conditions of the sows (Hemsworth et al., 1978). Therefore, an on average lack of difference concerning reproductive performance between methods as artificial insemination or natural mating under field conditions may be due to confounding influences of e.g. social conditions of sows. Studies concerning effects of boar stimulation on reproductive parameters, therefore, should include study of these possibly confounding influences and therewith may contribute to improved insemination strategies.

Reproductive performance in mature female pigs is predominantly determined by

farrowing rate and litter size. Differences in farrowing rate and litter size are mainly dependent on events during early pregnancy. Litter size is primarily determined by differences in embryonic mortality (Leymaster et al., 1986) whereas non-pregnancy after insemination mainly originates from fertilization failure or complete early embryonic death. Causes of embryonic mortality are still poorly understood. However, embryonic losses occurring after Day 11 of pregnancy have been hypothesized to be related with early within-litter embryonic diversity (reviewed by Pope et al., 1990). In this study, the question was raised whether and how environmental and/or management factors (such as insemination conditions), that are known to affect reproductive performance, influence paramters during early pregnancy and if so, how they influence these parameters.

Based on the above, in the first part of the present study, effects of insemination conditions and social conditions have been studied on early pregnancy. Emphasis has been given to effects on within-litter embryonic diversity because of its suggested relevance for embryonic mortality. Inconsistent results of the first experiments made clear that it would be necessary to assess the moment and duration of ovulation. Therefore, in the second part of this study, a method was developed and used to study the duration of ovulation as a cause of within-litter embryonic diversity under physiological conditions.

2. EFFECTS OF INSEMINATION CONDITIONS ON REPRODUCTIVE PARAMETERS DURING EARLY PREGNANCY DIFFER BETWEEN EXPERIMENTS

Experiments concerning effects of boar stimuli around insemination show that specific reproductive processes in gilts and sows can be affected (see Chapter 1). Both external stimulation (olfactory, tactile, visual and auditory) and internal stimulation (tactile and chemical stimulation by sperm plasma) are able to affect one or more of the following reproductive processes: standing response, sperm transport and ovulation. Effects on one or more of these processes subsequently may affect reproductive performance during pregnancy (fertilization rate, embryonic mortality) and at farrowing (farrowing rate and litter size).

In the present study, two experiments have been performed to evaluate effects of boar stimulation around insemination on reproductive parameters during early pregnancy, with emphasis on embryonic diversity (results have been presented in Chapters 2 - 5). Two insemination conditions were created, a stimulus-rich (AI + additional contact with a vasectomized boar) and a stimulus-poor (AI) insemination. The use of artificial insemination was chosen to create standard conditions concerning the number of sperm cells inseminated and the genotype of the boar. The stimulus value of vasectomized boars is considered equivalent to that of intact boars, except for differences in the composition of semen, such as

oestrogens (Claus et al., 1987; Chapter 1).

Despite similar conditions in the two experiments, socially restricted gilts showed a low pregnancy rate (on average 71%) and high embryonic mortality rate (on average 33%) in the first experiment and high pregnancy rate (on average 90%) and low embryonic mortality rate (on average 15%) in the second experiment. This might be related with experimental season, since reproductive performance is generally lower during summer (Experiment I) than during winter (Experiment II) according to Claus et al. (1985). The poor reproductive performance in Experiment I was accompanied by a higher percentage of gilts with a duration of oestrus of less than two days (64%; unpublished result) in non-stimulated gilts, compared to only 27% of similarly treated gilts in Experiment II. Causes of this difference in the duration of oestrus between Experiment I and II are difficult to pinpoint, since many factors such as season, degree of boar contact, levels of stress-related hormones and genetic effects (Willemse and Boender, 1966; Liptrap, 1970; Steinbach, 1976; Hemsworth et al., 1984) all may contribute to differences in the duration of oestrus. Since the moment of ovulation is associated with the duration of oestrus (Chapter 6; Wagner-Rietschel, 1991) and optimal time of insemination is dependent on the moment of ovulation (Willemse and Boender, 1967; Helmond et al., 1986), knowledge concerning the causes of the variability in the duration of oestrus may contribute to a better timing of insemination.

Effect of Insemination Conditions

Duration of Oestrus and Accessory Sperm Count

Boar stimulation significantly increased the number of gilts with a short duration of oestrus in Experiment II (Chapter 5; 27% vs 55%, $P<0.10$), but not significantly in Experiment I (unpublished result; 64% vs 80%, $P>0.10$). A shorter duration of oestrus suggests an advanced moment of ovulation due to boar stimulation, since ovulation normally occurs at a relatively fixed moment in oestrus independent of the length of the oestrous period (at approximately two-thirds of the duration of oestrus, Chapter 6). This shorter duration of oestrus as a result of boar stimulation, therefore, would correspond with results concerning advancement of ovulation due to boar stimulation at onset of oestrus (Chapter 1; Signoret et al., 1972; Brutgans, 1983; Weitze et al., 1990a, b, c). In Experiment I, a high percentage of non-stimulated gilts already had a short duration of oestrus. Consequently, in most gilts boar stimulation was applied relatively shortly before ovulation. Weitze et al. (1990a) showed that effects of boar stimulation on the moment of ovulation are less prominent when treatments were applied not at onset of oestrus but 16 hours thereafter. This may explain the lack of effect of boar stimulation on the duration of oestrus in Experiment I.

The recovery of pre-hatching embryos in Experiment II allowed for the assessment of the number of accessory sperm cells in the zona pellucida. In these embryos, boar stimulation

significantly increased the accessory sperm count, which suggests an improved sperm transport due to boar stimulation (see Chapter 1).

In summary, boar stimulation after AI increased the accessory sperm count, indicating improved sperm transport in Experiment II, and it caused a reduction in the duration of oestrus, indicating advancement of ovulation in Experiment II but not in Experiment I. Furthermore, reproductive performance expressed as pregnancy rate differed between the two experiments. Knowledge of the moment of ovulation in these animals might have contributed to an explanation of these variable results.

Embryonic Diversity

Causes of embryonic mortality in the sow are poorly understood. From 1982 onwards, experiments have been performed to substantiate the hypothesis that within-litter embryonic diversity is of major importance for embryonic mortality between Days 11 and 16 of pregnancy. In these experiments, either asynchronous embryo transfer was used to create embryonic diversity and/or a (partly) asynchronous uterine environment (e.g. Pope et al., 1982; Pope et al., 1986a; Geisert et al., 1991) or oestrogen administration was used to create an advanced uterine environment (e.g. Geisert et al., 1982; Morgan et al., 1987a, b; Gries et al., 1989; Geisert, 1991; Pope et al., 1986b). It was concluded that the relation between within-litter embryonic diversity and embryonic mortality is based on the mechanism that all embryos should develop synchronously with their uterine environment to survive. An advanced uterine environment, induced by either the more developed embryos in a litter (mimicked by asynchronous transplantation) or the uterus itself (mimicked by oestrogen injections), will cause the death of the less developed embryos. The tolerance level for asynchrony possibly varies during pregnancy, but seems low around Day 10 of pregnancy (Dziuk, 1987).

Under physiological conditions, within-litter diversity in pigs has been reported from the early embryonic phase (Xie et al., 1990a), throughout pregnancy (e.g. Perry and Rowlands, 1962; Anderson, 1978; Papaioannou and Ebert, 1988; Pusateri et al., 1990; Van der Lende, 1989) up to the late foetal stage (Van der Lende, 1989). Only one experiment has been performed to investigate the relation between early embryonic diversity and embryonic mortality under physiological conditions. Day 6 embryos were divided in 2-4 development groups and these groups were returned to ligated parts of a recipient uterus. At Day 13-15, embryonic mortality rate was negatively related with development at Day 6 (Wilmut et al., 1985). However, the authors did not present data on a relation between embryonic diversity and embryonic mortality per sow. Therefore, at this moment it is still unclear whether this relation is an explanation for differences in embryonic mortality between sows. In Chinese Meishan pigs, low embryonic mortality rates (Bidanel and Legault, 1986) have been related to lower embryonic diversity than in Large White sows (Bazer et al., 1988). However, Wilmut et al. (1990), Anderson et al. (1991) and Soede et al. (1992) did not find differences in early

embryonic diversity. To justify the existence of a relation between embryonic diversity and embryonic mortality based on differences between Meishan and Large White pigs is arguable, since Meishan pigs and Large White pigs differ in many aspects.

The rate of early embryonic mortality, both before and after Day 11 of pregnancy, greatly differs between experiments (Pope and First, 1985). Since embryonic diversity is expected to affect embryonic mortality only after Day 11 (Pope et al., 1990; Geisert et al., 1991), the contribution of embryonic diversity to embryonic mortality presumably differs between groups of sows.

Based on the above, the two experiments with varying insemination conditions (stimulus-rich vs stimulus-poor) and social conditions (socially-restricted vs socially-non-restricted) have been performed to elucidate whether or not differences in within-litter embryonic diversity between sows can be induced by environmental conditions which are known to affect embryonic mortality rate or litter size (Chapters 4 and 5). In these experiments, effects of boar stimulation on within-litter embryonic diversity were inconsistent. In the first experiment, within-litter embryonic diversity between Days 9 and 11 of pregnancy was increased by boar contact in the socially restricted gilts and decreased in the socially non-restricted gilts (Chapter 4). However, in the second experiment, embryonic diversity was not affected by boar stimulation in socially restricted gilts. The effects on embryonic diversity as found in the first experiment suggested that embryonic mortality after Day 11 is more likely to occur in socially restricted gilts with boar contact and in socially non-restricted gilts without boar contact. Interestingly, this would correspond with results as found by Hemsworth et al. (1978) concerning litter size using comparable experimental conditions.

In conclusion, boar stimulation may affect embryonic diversity and thus embryonic mortality, but only under specific conditions. The exact nature of these conditions is unknown. However, any factor causing differences in embryonic diversity between litters (see paragraph 4, page 127) may be of importance. To investigate effects of boar stimulation on within-litter embryonic diversity, it seems worthwhile to focus on causes of embryonic diversity, in order to avoid possible confounding influences during early pregnancy.

Interaction between Insemination Conditions and Social Conditions

Effects of boar stimulation on reproductive performance may depend on the social conditions of the sows, as was pointed out before (General Discussion, page 119). Therefore, in a first experiment, effects on early pregnancy and embryonic diversity were studied in socially non-restricted and socially restricted gilts (pairwise housed gilts with additional contact with gilts and a boar or individually housed gilts without these contacts, respectively) (Chapters 2 - 4). In the second experiment, similar insemination conditions were studied in socially restricted gilts only (Chapter 5).

In Experiment I, socially restricted gilts had a poor puberty attainment and a lower pregnancy rate (Chapter 2). Furthermore, in gilts that were given boar stimulation around insemination, socially restricted gilts showed a disturbed introductory sexual behaviour towards the boar (Chapter 3) and responded differently to the boar regarding embryonic diversity (Chapter 4; General Discussion, page 123). Effects such as these on puberty attainment, sexual behaviour and pregnancy rate have been knonw to occur as a result of social restriction (Prunier and Etienne, 1984; Hemsworth and Barnett, 1990; Hughes et al., 1990) and have been related with the existence of a chronic stress response in these gilts (Barnett et al., 1981). Stress-related hormones may interfere with all three levels of the hypothalamo-pituitary-gonadal axis and consequently affect reproduction traits (Rivier and Rivest, 1991).

3. DURATION OF OVULATION

Based on the experiments concerning influences of boar stimulation on reproductive parameters during early pregnancy, no definite conclusion could be drawn concerning the exact influence of boar stimulation on early pregnancy and embryonic diversity. The results made clear that effects of boar stimuli on reproductive performance should be studied more directly. The most direct parameters of interest are the moment of ovulation (ovulation of the first oocyte) and the duration of ovulation (interval between the ovulation of the first and the last oocyte) for each individual animal. These parameters were supposed to be of interest because of three reasons. First, seemingly inconsistent results of boar stimulation between experiments as found in the present study might have originated from effects on the moment and duration of ovulation. Knowledge of the moment and duration of ovulation contributes to a better understanding of the nature of the effects. Second, the moment of ovulation was reported to be affected by boar stimuli (Signoret et al., 1972; Weitze et al., 1990a, b, c). Effects on the duration of ovulation were also reported (Signoret, 1972), but were based on an average duration of ovulation in a group of sows. Third, the duration of ovulation seems a major cause of embryonic diversity (Pope et al., 1988; Xie et al., 1990a, b).

Assessment by transrectal ultrasonography

Estimates of the duration of ovulation in individual animals have been made by peritoneal cannulation (Betteridge and Raeside, 1962) or by repeated laparoscopy (Burger, 1952; Brüssow et al., 1990a, b). Peritoneal cannulation was not successful and continuous laparoscopy requires a lengthy surgery under general anaesthesia which may influence the process of ovulation. Therefore, in the present study, a non-invasive method to study the duration of ovulation was developed. Transcutaneous ultrasonography has been proven to be

an appropriate method to assess the moment of ovulation (Weitze et al., 1989, 1990a, b, c). However, to determine the duration of ovulation, the number of preovulatory follicles on both ovaries needs to be assessed repeatedly and accurately. In own preliminary studies, neither transcutaneous nor transvaginal ultrasonography gave satisfactory results. In later studies, transrectal ultrasonography under manual guidance was performed (see Appendix 1). Compared with the corpora lutea count, the deviation of the maximum follicle count was zero or only one in 77% of the ovaries and the deviation was greater than two in only 5% of the ovaries (Chapter 6+7). Consequently, it was assumed that the duration of ovulation could be determined accurately using transrectal ultrasonography. Whether the process of ovulation is influenced by transrectal ultrasonography or not can not be investigated directly since no other non-invasive methods are available to study onset and duration of ovulation. However, other reproductive parameters such as fertilization rate, accessory sperm count, embryonic development and embryonic diversity at Day 4 after insemination were not influenced by transrectal ultrasonography, as compared to a non-scanned control group (Chapter 7). It was concluded that transrectal ultrasonography is an appropriate method to study moment and duration of ovulation. The method can only be applied in sows with an internal pelvic diameter allowing rectal exploration. Therefore, in experiments studying the duration of ovulation, sows instead of gilts were used.

Using transrectal ultrasonography, the duration of ovulation was assessed in three groups of sows, which differed in social-, insemination-, synchronization-conditions and conditions during scanning (Chapters 6 and 7). The duration of ovulation significantly differed both within groups and between groups. In the sows, the minimum duration of ovulation was 0.8 hours and the maximum duration of ovulation was 7 hours. Since the three groups of sows differed in many respects, at this moment it is not clear which factor(s) contribute to differences in the duration of ovulation between groups of sows, nor within groups of sows.

Effect of insemination conditions

The specific influence of insemination conditions on the process of ovulation (moment and duration) has not been investigated thoroughly in the present study. Nevertheless, in oestrus-synchronized sows, insemination conditions were varied by application of either double artificial insemination (22 hours and 30 hours after hCG-injection) or no insemination (Chapter 7). Under these conditions, insemination neither influenced the moment of ovulation, nor the duration of ovulation (on average 2.4 hours). Weitze et al. (1990b) showed that, compared to non-insemination, insemination with physiological saline at onset of oestrus advanced ovulation by approximately 7 hours, from 45 to 38 hours after onset of oestrus; insemination with sperm plasma advanced ovulation by another 8 hours. Application of the same treatments at 16 hours after onset of oestrus showed that physiological saline was ineffective in advancing ovulation

and that sperm plasma advanced it by 10 hours. Furthermore, the complete range of stimuli present during mating at onset of oestrus and 6 hours thereafter, has been found to affect both the moment and duration of ovulation (Signoret et al., 1972). In conclusion, these results suggest that to achieve advancement of ovulation and possibly a reduction in the duration of ovulation, boar stimuli should be applied at a relatively early stage of oestrus and/or boar stimulation should be intense.

Relation with within-litter embryonic diversity

Attempts to quantify and influence the duration of ovulation (Chapters 6 and 7) were undertaken because of the hypothesized relation between the duration of ovulation and within-litter embryonic diversity. A few remarks must be made about the assumptions and results of the experiments leading to this hypothesis to scrutinize their rightness.

- Within litters, pre-ovulatory oocyte maturation (Xie et al., 1990a), pre-ovulatory follicular development (Xie et al., 1990c) and zygotic development (Xie et al., 1990a) are negatively skewed and related to the negatively skewed ovulation (Xie et al., 1987; Pope et al., 1988). Based on findings from laparotomy performed once in 52 gilts at 34 hours after onset of oestrus, Pope et al. (1988) concluded that 70% of the follicles rupture almost simultaneously and the remaining follicles rupture over a longer period of time. However, using this method, neither individual variation in the duration of ovulation, nor individual variation in the course of ovulation can be detected. Furthermore, similarities between the skewed development of oocytes, follicles and zygotes as described by Xie et al. (1990a, c) probably are not very exact. The latter because all parameters have discrete distributions of development in which 1) a small number of classes is included; 2) the classes encompass different periods of time, and 3) also start- and end-phases of development are included. Although the existence of within-sow diversity in developmental stages of oocytes, follicles and zygotes is not argued, the proposed 75:25 majority:minority distribution may not be a true reflection of the degree of diversity in development.

- Within sows, the moment of ovulation and the ranking of embryonic development at Day 4 are related and , furthermore, ranking of Day 4 development has been related with ranking of Day 11 development (Xie et al., 1990b). In hCG-treated sows (n=12), either the first ovulating oocytes (39 hours after hCG) or the last ovulating oocytes (42 hours after hCG) were removed from the ovary, stained, placed in the oviduct and recovered after 75 hours. Within gilts, the first ovulating follicles were the further developed embryos and the last ovulating follicles were the less developed embryos. In additional gilts, 'younger' and 'older' Day 4 embryos were sorted based on cell number and placed in one of the two ligated horns of a

non-pregnant recipient. At Day 12, the younger embryos were still the less developed embryos. Therefore, the ranking of the moment of ovulation remained detectable in embryonic development up to Day 11 after insemination. In these experiments, no information is presented about differences in the degree of diversity between litters.

- A reduction in the duration of ovulation is related with a reduction in embryonic diversity at Day 11 after insemination (Pope et al., 1988). Of gilts in the process of ovulation at surgery, the 2-4 remaining follicles were destroyed by electrocautery. In control gilts, electrocautery of ovarian stroma was performed immediately after ovulation. At Day 11 of pregnancy, taking into account only gilts with 100% embryonic survival, control gilts showed higher embryonic diversity than the treatment group. The latter was due to the presence of less developed embryos in the control gilts.

In conclusion, within litters, the ranking of ovulation of oocytes is related with the ranking of early embryonic development (Xie et al., 1990a, b, c) and results of Pope et al. (1988), suggest that this relation between the moment of ovulation and the ranking of embryonic development may explain differences in embryonic diversity between litters. In those experiments, no information is presented about differences in the degree of embryonic diversity between litters. In a review, Pope et al. (1990) stated that, after ovulation, SLA-genotype (Ford et al., 1988) may contribute to embryonic diversity, but factors such as: time of fertilization, sex-linked genes, oviductal transport, time of hatching from the zona pellucida and uterine location are of minor importance.

In the present study, assessment of the duration of ovulation in individual sows using transrectal ultrasonography has given the opportunity to relate the duration of ovulation with early within-litter embryonic diversity. In total, 28 sows with a duration of ovulation between 1 and 4 hours were slaughtered between 90 and 120 hours after ovulation. Embryonic diversity was expressed as the within-litter standard deviation of development and as the range of within-litter embryonic development. Embryonic diversity was large compared to the duration of ovulation, which was maximal 4 hours. Even the smallest range in number of cell cycles within a litter (1.2) corresponds with an 18 hours difference in age (the average duration of the first five cell cycles is approximately 12 hours (Hunter, 1973)). Under the described conditions, between sows, no relation between the duration of ovulation and early embryonic diversity was detected (Chapter 6; Figure 3, Chapter 7; Figure 2). Since these results do not support those of Pope et al. (1988) and since differences in embryonic diversity between litters were large, the question was raised which factors might have caused differences in embryonic diversity between litters.

4. PHYSIOLOGICAL CAUSES OF DIFFERENCES IN EMBRYONIC DIVERSITY BETWEEN LITTERS

In chronological order, differences in embryonic diversity between litters may result from differences in variation of: (a) oocyte maturation, (b) duration of ovulation, (c) duration of oocyte transport to the site of fertilization, (d) duration of fertilization (interval between the fertilization of the first oocyte and the last oocyte) and (e) rate of early embryonic development. Additionally, fertilized oocytes originating from either the left or the right ovary, are separated until approximately Day 5 of pregnancy (Dhindsa et al., 1967). Therefore, even within sows, embryonic diversity between embryos originating from the left side and the right side may be influenced by oocyte maturation, moment of ovulation, oocyte transport and moment of fertilization. As an extreme example, unilateral fertilization may occur as a result of a disproportionate distribution of sperm over the two uterine horns (Hancock, 1962).

The possible influence of these factors will be outlined.

a. Oocyte maturation.

Differences in within-litter variation in pre-ovulatory oocyte maturation and follicular morphology have been reported between naturally cyclic and PMSG/hCG-treated prepubertal gilts (Hunter and Wiesak, 1990; Wiesak et al., 1990). Within sows, a skewed distribution of oocyte maturation has been related with a skewed distribution of ovulation (Xie et al., 1990a). However, the existence of the latter relation, based on skewness-comparisons, may not be valid (see page 126). It might be hypothesized that differences in oocyte maturation are still noticeable after ovulation and may hence influence early embryonic diversity.

b. Duration of ovulation.

Differences in the duration of ovulation between sows have been reported using continuous laparoscopy (Brüssow et al., 1990a, b) and using continuous transrectal ultrasonography (Chapters 6 and 7). Brüssow et al. (1990a, b) reported a duration of ovulation between 6 and more than 9 hours, whereas in the present study, ovulation lasted 1 to 7 hours. In the present study, a duration of ovulation between 1 and 4 hours was not related with differences in early embryonic diversity. If transrectal ultrasonography caused a reduction in the duration of ovulation masking a relation with embryonic diversity, embryonic diversity should have expected to be higher in non-scanned sows. However, this was not the case (Chapter 7). Therefore, the duration of ovulation does not seem an important determinant for differences in early embryonic diversity between sows.

c. Oocyte transport to the site of fertilization.

Depending on the oestrogen-progesterone ratio, both oocytes and sperm cells are

transported to the site of fertilization. After ovulation, gradually oestrogen dominance changes into progesterone dominance after which both fertilized and unfertilized oocytes are transported through the isthmus to the utero-tubal junction. Variable progesterone-oestrone ratios (Hunter, 1972) and concentrations of PGF2α (Rodriquez-Martinez and Einarsson, 1985) may affect oviduct motility, which may consequently affect transport of gametes to the site of fertilization. Preovulatory follicle fluid contains large quantities of PGF2α (reviewed by Ainsworth et al., 1990) which enters the infundibulum after ovulation, but is thought to be predominantly displaced into the peritoneal cavity (Hunter et al., 1990). The increasing amounts of PGF2α, released from follicle fluid might cause differences in oviduct motility during the process of ovulation and therewith increase oocyte transport from the first ovulating oocyte up to the last ovulating oocyte. This consequently would decrease the difference in embryonic diversity which is due to difference in the duration of ovulation, and would (partly) explain the lack of relation between the duration of ovulation and embryonic diversity as found in the present study.

d. Duration of fertilization.

Pope et al. (1990) asserted that, within-litters, time of fertilization fails to influence embryonic diversity. However, for example, artificial insemination with a low concentration of sperm cells significantly reduces fertilization rate (Baker et al., 1968). It seems logical to expect that the presence of only few sperm cells at the site of fertilization, by whatever cause, not only reduces fertilization rate but also increases the duration of fertilization from the first up to the last oocyte. Dziuk (1987) suggested that, under suboptimal conditions, duration of fertilization may be as much as 8 to 10 hours. An extended duration of fertilization consequently may overrule effects of the duration of ovulation (1 to 7 hours; Chapters 6 and 7) on within-litter embryonic diversity.

In the present study, the number of accessory sperm cells have been considered as a measure for sperm transport. In Figure 1, the residuals of the within-litter embryonic diversity corrected for average embryonic development ($P=0.001$) have been plotted against the average accessory sperm count per litter for the sows of which the duration of ovulation was assessed (Chapter 6; Group A ($n=13$), Chapter 7: Group Ovul+ ($n=14$)). In these sows, an increasing sperm count is associated with a reduction in embryonic diversity ($P=0.014$). This relation was similar for the spontaneously ovulating sows (Chapter 6) and those with induced ovulation (Chapter 7). From this Figure 1, it seems plausible that a low average sperm count is associated with an extended duration of fertilization, which consequently affects embryonic diversity. In these sows, duration of ovulation was not associated with embryonic diversity, not even in the 11 sows with an average sperm count of more than 20. Therefore, in these sows, duration of fertilization seems a more important determinant for embryonic diversity than duration of ovulation. The present results were obtained using the following insemination

strategies: sows were either artificially inseminated on every day of standing heat (Chapter 6) or both at 22 hours and 30 hours after hCG-injection (Chapter 7). These strategies rendered good results in terms of fertilization rate (on average, 96% and 91%, respectively). Furthermore, sperm count was not affected by scanning (see Chapter 7). Consequently, variation in sperm counts between litters such as presented in Figure 1 seem to be valid under common conditions.

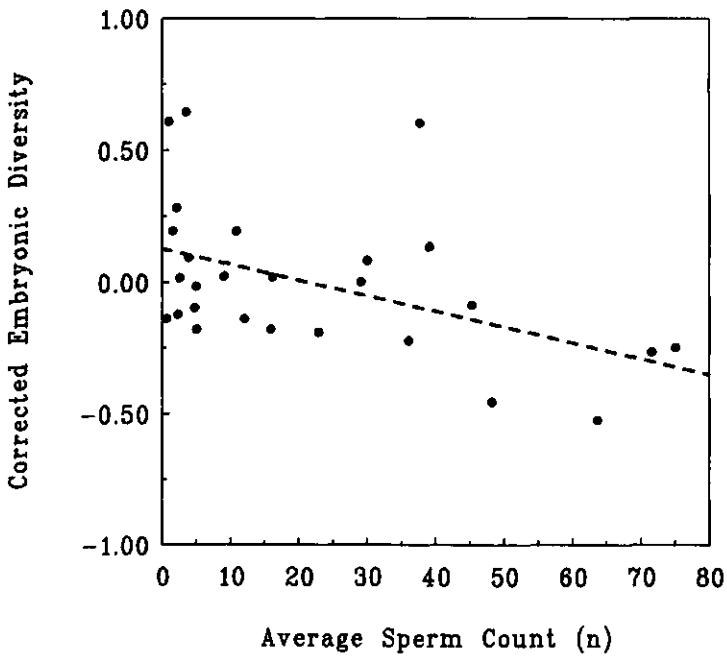

Figure 1. Relation between the average number of accessory sperm cells per litter and the residuals of the within-litter embryonic diversity (s.d. of cell cycles) corrected for average embryonic development from sows of Chapter 6 ($n=13$, Group A) and Chapter 7 ($n=14$, Group Ovul+) in which the duration of ovulation was assessed (● one litter, ---- $Y=0.128-0.006*X$; $R^2=0.40$; $P=0.014$).

e. Rate of early embryonic development.

Genetic factors such as breed (Bazer et al., 1988) and/or SLA-genotype (Ford et al., 1988) have been found to affect differences in within-litter embryonic diversity. Furthermore, variable concentrations of oviductal and uterine components might influence differences in early embryonic development between litters, as is often hypothesized based on results of in vitro culture of embryos (Bavister, 1988).

From this outline, it is clear that within-litter embryonic diversity may differ between sows, independently of the duration of ovulation. In our study, especially the duration of fertilization seemed of importance. Therefore, the relation between the duration of ovulation and early embryonic development, both within (Xie et al., 1990b) and between litters (Pope et al., 1988) can only be found when the moment of fertilization is closely associated with the moment of ovulation. Xie et al. (1990a, b) suggested the existence of this close relation between ovulation and fertilization in their experiments, based on a slightly shifted distribution of zygotic development following an artificially induced shift in ovulation by aspiration of follicles (Xie et al., 1990a). They did not report any sperm counts in their experiments. Our results show that, even when using a presumed optimal insemination strategy, sperm counts may often be low and are related to embryonic diversity.

A lack of relation between the duration of ovulation and early within-litter embryonic diversity between litters as found in the present study (Chapters 6 and 7), consequently suggests that the duration of ovulation is not the predominant factor in explaining differences in embryonic mortality between sows. A reduced sperm transport and/or a suboptimal insemination strategy causing a lengthy duration of fertilization may be a more important factor.

Summarizing, in literature it was hypothesized that embryonic diversity is predominantly related to the duration of ovulation, whereas the present results argue this hypothesis. As was outlined, several other physiological causes of differences in embryonic diversity can be indicated, namely differences in oocyte maturation, oocyte transport, the duration of fertilization and the rate of embryonic development. This sheds new light on the results of Experiments I and II, from which it was concluded that insemination conditions (a stimulus-poor insemination vs a stimulus-rich insemination) and social conditions of sows (socially-rich vs socially-poor) may affect embryonic diversity (Chapter 4, paragraph 2, page 123).

Effects of boar stimuli on embryonic diversity (Chapter 4) presumably are not related to effects on the duration of ovulation (Chapter 6 and Chapter 7), but may be related to effects on oocyte transport and the duration of fertilization. Oestrogens, present in boar semen, stimulate the synthesis and release of PGF2α, which affect oviduct motility and therewith gamete transport to the site of fertilization (see Chapter 1), consequently affecting both oocyte transport and the duration of fertilization. The latter is, furthermore, indicated by the increased accessory sperm count due to boar stimulation (Chapter 5) in combination with the existence of a negative relation between the accessory sperm count and embryonic diversity (this paragraph, Figure 1).

Effects of social conditions on embryonic diversity as described in Chapter 4, possibly

are related to the stressful conditions of social restriction. In experiments in which ACTH was administered to mimic stressful conditions, both increased cortisol levels and inhibition of the LH surge was found (Barb et al., 1982) and ovulation was disturbed (Barb et al. 1982; Hennessy and Williamson, 1983). Furthermore, in vitro studies showed that cortisol affected steroid secretion from developing pig follicles (Ryan et al., 1990). Therefore, stress-related hormonal changes may affect both oocyte and follicular maturation and consequently affect embryonic diversity.

Effects of boar stimulation around insemination on reproductive performance in terms of litter size may be related with effects on embryonic diversity as outlined above. Furthermore, reproductive performance in terms of pregnancy rate and litter size may be affected by the advanced ovulation and improvement of sperm transport (Chapter 1 and Chapter 5). Especially in case of a suboptimal timing of insemination relative to ovulation, these effects on sperm transport and ovulation may contribute to a synchronized presence of the male and female gametes at the site of fertilization. This may subsequently affect reproductive performance. Willemse and Boender (1967) showed that a suboptimal timing of insemination resulted in a decreased litter size and Helmond et al. (1986) reported a decreased fertilization rate and an increased early embryonic mortality rate. Furthermore, results from the present study (paragraph 4, page 130) make plausible that, even when using a presumed optimal insemination strategy, sperm transport may be suboptimal.

In conclusion, effects of boar stimulation around insemination on litter size as reported in literature, may be related to effects on embryonic diversity (Chapter 4). These effects on embryonic diversity presumably are not related to effects on the duration of ovulation since no relation was found between the duration of ovulation and embryonic diversity (Chapter 6 and Chapter 7). However, embryonic diversity appeared to be related to the duration of fertilization (see Figure 1) and may consequently be related to embryonic mortality. Further research is needed to substantiate this. Effects of boar stimulation on embryonic mortality may, furthermore, be related with effects on the onset of ovulation, causing a more synchronized presence of the male and female gametes at the site of fertilization. In further studies concerning boar stimulation, in studies on insemination strategies and in other studies in which the exact moment of ovulation needs to be assessed, the technique of transrectal ultrasonography, developed to study the duration of ovulation in pigs, is an indispensable tool.

CONCLUSIONS

Boar stimuli immediately after insemination affect the duration of oestrus, the number of accessory sperm cells, embryonic development and embryonic diversity. This suggests that boar stimuli affect the process of ovulation and the transport of sperm. By unknown causes, the influence of boar stimuli varies between experiments.

Effects of boar stimuli around insemination on litter size, as reported in literature, seem to originate from differences in within-litter embryonic diversity and associated changes in embryonic mortality.

Transrectal ultrasonography is a good method to count the number of follicles repeatedly and accurately and monitor the moment of first ovulation and the duration of ovulation. There are no indications that the process of ovulation and sperm transport are influenced by the procedure.

The duration of ovulation, as assessed by transrectal ultrasonography, varied between sows (from 1 to 7 hours) and varied between groups of sows (from 1.8 ± 0.6 to 4.6 ± 1.7 hours). The cause(s) of this variability is (are) unknown.

The duration of ovulation (varying between 1 and 4 hours) was not related to embryonic diversity. Consequently, the duration of ovulation may neither be an important determinant for embryonic diversity, nor for associated embryonic mortality.

Differences in within-litter embryonic diversity are related to the average number of accessory sperm cells. This suggests that the duration of fertilization is an important determinant for embryonic diversity and associated embryonic mortality.

REFERENCES

Ainsworth, L., Tsang, B.K., Downey, B.R. and Marcus, G.J., 1990. The synthesis and actions of steroids and prostaglandins during follicular maturation in the pig. J. Reprod. Fertil., Suppl. 40: 137-150.

Anderson, L.L., 1978. Growth, protein content and distribution of early pig embryos. Anat. Rec., 190: 341-353.

Anderson, L.H., Christenson, L.K. and Ford, S.P., 1991. Embryonic diversity and estrogen content of littermate embryos from Chinese (Meishan) and crossbred sows. J. Anim. Sci., 69 (Suppl. 1): 529. (Abstract).

Baker, R.D., Dziuk, P.J. and Norton, H.W., 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. J. Anim. Sci.,27: 88-93.

Barb, C.R., Kraeling, R.R., Rampacek, G.B., Fonda, E.S. and Kiser, T.E., 1982. Inhibition of ovulation and LH secretion in the gilt after treatment with ACTH or hydrocortisone. J. Reprod. Fertil., 64: 85-92.

Barnett, J.L., Cronin, G.M. and Winfield, C.G., 1981. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. Gen. Comp. Endocrinol., 44: 219-225.

Bavister, B.D., 1988. Role of oviductal secretions in embryonic growth in vivo and in vitro. Theriogenology, 29: 143-154.

Bazer, F.W., Thatcher, W.W., Martinat-Botte, F. and Terqui, M., 1988. Conceptus development in Large White and prolific Chinese Meishan pigs. J. Reprod. Fertil., 84: 37-42.

Betteridge, K.J. and Raeside, J.I., 1962. Observations on the ovary by peritoneal cannulation in pigs. Res. Vet. Sci., 3: 390-398.

Bidanel, J.P. and Legault, C., 1986. Experimental and prospective aspects of utilization of prolific Chinese pig breeds in Europe. Proc. 37th Meeting Eur. Assoc. Anim. Prod., Budapest, 1: 104-105 (Abstract).

Brüssow, K.P., Ratky, J., Kanitz, W. and Becker, F., 1990a. Determination of the duration of ovulation in gilts by means of laparoscopy. Reprod. Dom. Anim., 25: 184-190.

Brüssow, K.P., Ratky, J., Kanitz, W. and Becker, F., 1990b. The relationship between the surge of LH induced by exogenous GnRH and the duration of ovulation in gilts. Reprod. Dom. Anim., 25: 255-260.

Brutgans, Y.P., 1983. The role of boar semen plasma in fertilisation. Pig News and Inf., 4: 1178 (Abstract).

Burger, J.F., 1952. Sex physiology of pigs. Onderstepoort J. Vet. Res., 25 (Suppl. 1): 22-131.

Crabo, B.G., 1990. Preservation of boar semen: a worldwide perspective. Reprod. Dom. Anim., Suppl.1: 3-9.

Claus, R., Weiler, U. and Hahn, R., 1985. Influence of light and photoperiod on pig prolificacy. J. Reprod. Fertil., Suppl. 33: 185-197.

Claus, R., Hoang-Vu, C., Ellendorff, F., Meyer, H.D., Shopper, D. and Weiler, U., 1987. Seminal oestrogens in the boar: origin and functions in the sow. J. Steroid Biochem., 27: 331-335.

Dhindsa, D.S., Dziuk, P.J. and Norton, H.W., 1967. Time of transuterine migration and distribution of embryos in the pig. Anat. Rec., 159: 325-330.

Dziuk, P.J., 1987. Embryonic loss in the pig: an enigma. In: Manipulating pig production. J.L. Barnett, E.S. Batterham, G.M. Cronin, C.Hansen, P.H. Hemsworth, D.P. Hennessy, P.E. Hughes, N.E. Johnston and R.H. King (Editors). Australasian Pig Science Association, Werribee, Vic., pp. 28-39.

Ford, S.P., Schwartz, N.K., Rothschild, M.F., Conley, A.J. and Warner, C.M., 1988. Influence of SLA-haplotype on preimplantation embryonic cell number in miniature pigs. J. Reprod. Fertil., 84: 99-104.

Geisert, R.D., Thatcher, W.W., Roberts, R.M. and Bazer, F.W., 1982. Establishment of pregnancy in the pig. III. Endometrial secretory response to estradiol valerate administration on Day 11 of the estrous cycle. Biol. Reprod., 27: 957-965.

Geisert, R.D., Morgan, G.L., Zavy, M.T., Blair, R.M., Gries, L.K., Cox, A. and Yellin, T., 1991. Effect of asynchronous transfer and oestrogen administration on survival and development of porcine embryos. J. Reprod. Fertil., 93: 475-481.

Gries, L.K., Geisert, R.D., Zavy, M.T., Garvett, J.E. and Morgan, G.L., 1989. Uterine secretory alteration coincident with embryonic mortality in the gilts after exogenous estrogen administration. J. Anim. Sci.,

67: 276-284.

Hancock, J.L., 1962. Fertilization in farm animals. Anim. Breed. Abstr., 30: 285-310.

Helmond, F., Aarnink, A. and Oudenaarden, C., 1986. Periovulatory hormone profiles in relation to embryonic development and mortality in pigs. In: Embryonic mortality in farm animals. J.M. Sreenan and M.G. Diskin (Editors), Martinus Nijhoff, Dordrecht, The Netherlands, pp. 119-125.

Hemsworth, P.H. and Barnett, J.L., 1990. Behavioural responses affecting gilt and sow reproduction. J. Reprod. Fertil., Suppl. 40: 343-354.

Hemsworth, P.H., Beilharz, R.G. and Brown, W.J., 1978. The importance of the courting behaviour of the boar on the success of natural and artificial mating. Appl. Anim. Ethol., 4: 341-347.

Hemsworth, P.H., Cronin, G.M., Hansen, C. and Winfield, C.G., 1984. The effect of two oestrus detection procedures and intense boar stimulation near the time of oestrus on mating efficiency of the female pig. Appl. Anim. Behav. Sci., 12: 339-347.

Hennessy, D.P. and Williamson, P., 1983. The effects of stress and ACTH administration in hormone profiles, oestrus and ovulation in pigs. Theriogenology, 20: 13-26.

Hughes, P. e., Pearce, G.P. and Patterson, A.M., 1990. Mechanisms mediating the stimulatory effects of the boar on gilt reproduction. J. Reprod. Fertil., Suppl. 40: 323-341.

Hunter, R.H.F., 1972. Local action of progesterone leading to polyspermic fertilization in pigs. J. Reprod. Fertil., 31: 433-444.

Hunter, R.H.F., 1973. Chronological and cytological details of fertilization and early embryonic development in the domestic pig, *Sus scrofa*. Anat. Rec., 178: 169-186.

Hunter, M.G. and Wiesak, T., 1990. Evidence for and implications of follicular heterogeneity in pigs. J. Reprod. Fertil., Suppl. 40: 163-177.

Hunter, R.H.F., 1990. Fertilization of pig eggs in vivo and in vitro. J. Reprod. Fertil., Suppl. 40: 211-226.

Johnston, D.R., 1963. The history of human infertility. Fertil. Steril., 14: 261-272.

Levine, S., Coe, C. and Wiener, S.G., 1989. Psychoneuroendocrinology of stress: a psychobiological perspective. In: Psychoendocrinology. F.R. Brush and S. Levine (Editors). Academic Press, Inc., San Diego, USA, pp. 341-377.

Leymaster, K.A., Christenson, R.K. and Young, L.D., 1986. A biological model to measure uterine potential for litter size in swine. 3rd World Congr. Genet. Appl. Livest. Prod., XI. Genetics of reproduction, lactation, growth, adaptation, disease, and parasite resistance. Lincoln, Nebraska, pp. 209-214.

Liptrap, R.M., 1970. Effect of corticotrophin and corticosteroids on oestrus, ovulation and oestrogen excretion in the sow. J. Endocrinol., 47: 197-205.

Medvei, V.C., 1982. A history of endocrinology. MTP press ltd, Lancaster, England, 913 pp.

Morgan, G.L., Geisert, R.D., Zavy, M.T., Shawley, R.V. and Fazleabas, A.T., 1987a. Development of pig blastocysts in a uterine environment advanced by exogenous oestrogen. J. Reprod. Fertil., 80: 125-131.

Morgan, G.L., Geisert, R.D., Zavy, M.T. and Fazleabas, A.T., 1987b. Development and survival of pig blastocysts after oestrogen administration on Day 9 or Days 9 and 10 of pregnancy. J. Reprod. Fertil., 80: 133-141.

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

Perry, J.S. and Rowlands, I.W., 1962. Early pregnancy in the pig. J. Reprod. Fertil., 4: 175-188.

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

Pope, W.F., Maurer, R.R. and Stormshak, F., 1982. Survival of porcine embryos after asynchronous transfer. Proc. Soc. Exp. Biol. Med. 171: 179-183.

Pope, W.F., Lawyer, M.S., Nara, B.S. and First, N.L., 1986a. Effect of asynchronous superinduction on embryo survival and range of blastocyst development in swine. Biol. Reprod. 35: 133-137.

Pope, W.F., Lawyer, J.S., Butler, W.R., Foote, R.H. and First, N.L., 1986b. Dose-response shift in the ability of gilts to remain pregnant following exogenous estradiol-17ß exposure. J. Anim. Sci., 63: 1208-1210.

Pope, W.F., Wilde, M.H. and , S., 1988. Effect of electrocautery of nonovulated Day 1 follicles on subsequent morphological variation among Day 11 porcine embryos. Biol. Reprod., 39: 882-887.

Pope, W.F., , S., Broermann, D.M. and Nephew, K.P., 1990. Causes and consequences of early embryonic diversity in pigs. J. Reprod. Fertil., Suppl. 40: 251-260.

Prunier, A., Etienne, M., 1984. Effects of confinement on attainment of puberty in gilts. Ann. Réch. Vét., 15: 159-164.

Pusateri, A.E., Rothschild, M.F., Warner, C.M. and Ford, S.P., 1990. Changes in morphology, cell number, cell size and cellular estrogen content of individual litter mate pig conceptuses on days 9 to 13 of gestation. J. Anim. Sci., 68: 3727-3735.

Rivier, C. and Rivest, S., 1991. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biol. Reprod., 45: 523-532.

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

Ryan, P.L., King, G.J. and Raeside, J.I., 1990. Direct effects of cortisol on steroid production by granulosa cells from PMSG-induced follicles of prepubertal gilts. Anim. Reprod. Sci., 23: 75-86.

Signoret, J.P., Du Mesnil du Buisson, F. and Mauléon, P., 1972. Effect of mating on the onset and duration of ovulation in the sow. J. Reprod. Fertil., 31: 327-330.

Soede, N.M., Nieuwenhuis, C.C. and Van der Lende, T., 1992. Paired somite counts to assess the within-litter diversity in embryonic development at Day 17, 19 and 21 of pregnancy in Meishan and Yorkshire*Landrace crossbred gilts. Accepted for publication in Reprod. Dom. Anim.

Steinbach, J., 1976. Influence of the tropical climate on the fertility of pigs. Der Tierzüchter, 6: 272-274.

Van der Lende, T., 1989. Impact of early pregnancy on prenatal development in the pig. Thesis Agricultural University Wageningen, 151 pp.

Wagner-Rietschel, H., 1991. Studies on estrus and ovulation of sows using transcutaneous sonography. Thesis Veterinary University Hannover, Germany, 100 pp.

Weitze, K.F., Habeck, O., Willmen, T. and Rath, D., 1989. Detection of ovulation in the sow using transcutaneous sonography. Reprod. Dom. Anim., 24: 40-42.

Weitze, K.F., Rabeler, J., Willmen. T. and Waberski, D., 1990b. Interaction between inseminate, uterine and ovarial function in the sow. I. Influence of seminal plasma and oestrogens in the inseminate on intragenital sperm transport, time of ovulation and fertility results in gilts. Reprod. Dom. Anim., 25: 191-196.

Weitze, K.F., Lotz, J.H., Everwand, A., Willmen, T. and Waberski, D., 1990b. Interaction between inseminate, uterine and ovarial function in the sow. II. Investigations into the influencing of ovulation by the use of sperm free media. Reprod. Dom. Anim., 25: 197-204.

Weitze, K.F., Rath, D., Willmen, T., Waberski, D. and Lotz, J., 1990c. Advancement of ovulation in the sow related to seminal plasma application before insemination. Reprod. Dom. Anim., 25: 61-67.

Wiesak, T., Hunter, M.G. and Foxcroft, G.R., 1990. Differences in follicular morphology, steroidogenesis and oocyte maturation in naturally cyclic and PMSG/hCG-treated prepubertal gilts. J. Reprod. Fertil., 89: 633-641.

Willemse, A.H. and Boender, J., 1966. A quantitative and qualitative analysis of oestrus in gilts. Tijdschr. Diergeneesk., 91: 349-363.

Willemse, A.H. and Boender, J., 1967. The relation between the time of insemination and fertility in gilts. Tijdschr. Diergeneesk., 92: 18-34.

Wilmut, I., Haley, C.S., Ashworth, C.J., Aitken, R.P. and Ritchie, W., 1990. Embryo development and embryo transfer in Meishan and Large White pigs. Proc. 4th World Congr. Genet. Appl. Livest. Prod., p. 347-350.

Xie, S., Wilde, M.H., Nephew, K.P. and Pope, W.F., 1987. Skewed distribution of ovulation in gilts. J. Anim. Sci., 65 (Suppl.1): 414 (Abstract).

Xie, S., Broermann, D.M., Nephew, K.P., Bishop, M.D. and Pope, W.F., 1990a. Relationship between oocyte maturation and fertilization on zygotic diversity in swine. J. Anim. Sci., 68: 2027-2033.

Xie, S., Broermann, D.M., Nephew, K.P., Geisert, R.D. and Pope, W.F., 1990b. Ovulation and early embryogenesis in swine. Biol. Reprod., 43: 236-240.

Xie, S., Broermann, D.M., Nephew, K. P., Ottobre, J.S., Day, M.L. and Pope, W.F., 1990c. Changes in follicular endocrinology during final maturation of porcine oocytes. Dom. Anim. Endocrinol., 7: 75-82.

Appendix 1. TRANSRECTAL ULTRASONOGRAPHY

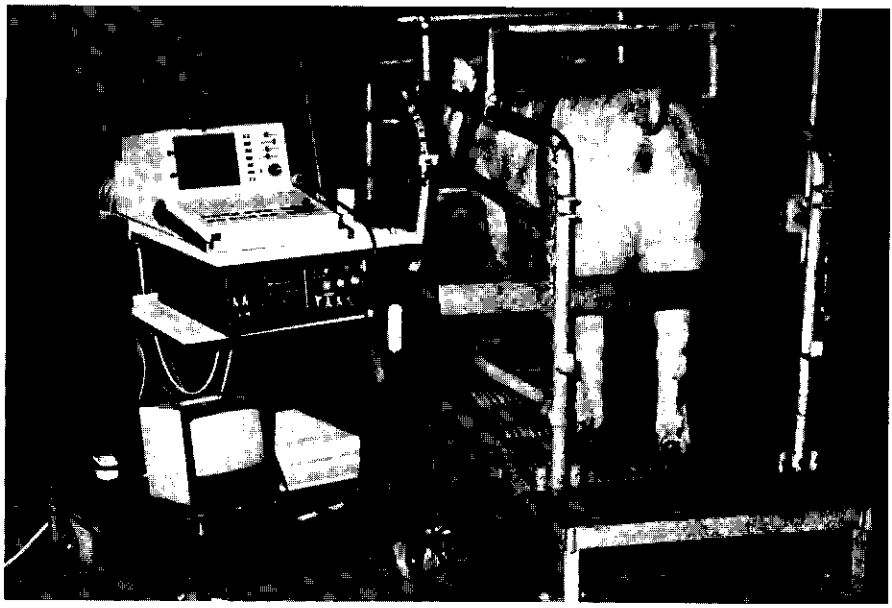

Figure 1. During scanning, sows were placed in metabolism cages to restrict their movements.

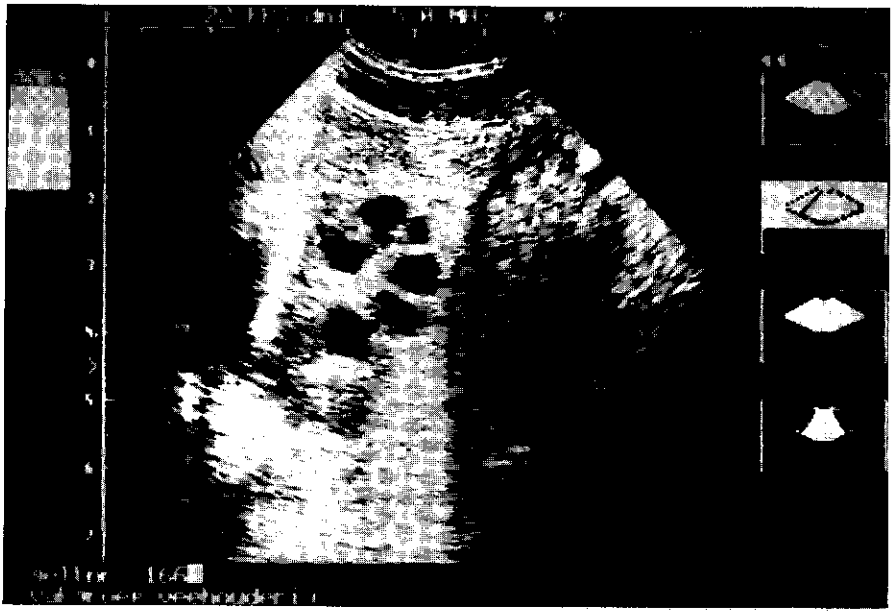

Figure 2. Scan-image of an ovary containing preovulatory follicles using an annular array sector scanner with a 5 MHz multiple scan angle transducer (Pie Medical b.v., Maastricht, The Netherlands).

SUMMARY

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Introduction

In pig husbandry, reproductive performance after either natural mating (NM) or artificial insemination (AI) is considered to be similar. However, possible differences in reproductive performance between AI and NM under experimental conditions have hardly been evaluated. Moreover, studies investigating effects of boar stimulation under experimental conditions (Chapter 1, review of literature), show that besides sperm cells, a whole range of stimuli emitted by the boar may affect specific reproductive processes, such as the standing response, sperm transport and the process of ovulation. These consequently may affect reproductive performance in terms of pregnancy rate and litter size. Both external stimuli (mainly olfactory and tactile) and internal stimuli (mainly components of seminal plasma) are of importance. In addition, from this review (Chapter 1), it is clear that effects of boar stimuli on litter size and pregnancy rate are dependent on the social conditions of the sows. These and other confounding factors may mask differences in reproductive performance obtained after AI and NM, respectively.

Differences in litter size as found after experimentally varied insemination conditions, may be due to differences in fertilization rate. Most probably, however, they are caused by differences in embryonic mortality. Generally, differences in litter size in pigs are caused by differences in embryonic mortality, i.e. mortality during the first month of pregnancy. Although the importance of embryonic mortality has been recognized for many years, causes of embryonic mortality still are poorly understood. In recent years, evidence has been presented that within-litter embryonic diversity is a major cause of embryonic mortality occurring after Day 11 of pregnancy. Around Day 11 of pregnancy, the more developed embryos in a litter synthesize oestrogens which modify the uterine environment. This modified uterine environment subsequently stimulates the development of these more developed embryos. However, a part of the less developed embryos will be unable to develop in this changed environment and will die.

Effect of insemination conditions on early pregnancy

Based on the above, the hypothesis was tested whether insemination conditions affect reproductive parameters during early pregnancy, emphasizing possible effects on within-litter embryonic diversity. Accordingly, the first part of this thesis is focused on the study of the influences of boar stimuli around insemination on within-litter embryonic diversity and on other aspects of early pregnancy (such as embryonic mortality, pregnancy rate and duration of oestrus) (Chapter 2 - Chapter 5).

In a first experiment, insemination conditions were varied in gilts that were housed under different social conditions from 5 months of age onwards; either socially restricted (individual housing without boar contact) or socially non-restricted (pairwise housing plus additional boar contact once daily from 6 months of age onwards). Insemination conditions were applied at third oestrus after puberty attainment, and consisted of either a stimulus-rich insemination (AI plus 20 min contact with a vasectomized boar) or a stimulus-poor insemination (AI). The use of AI was chosen to create standard conditions concerning the number of sperm cells inseminated and the genotype of the boar. The stimulus value of a vasectomized boar is generally equivalent to that of intact boars (see Chapter 1). These gilts were slaughtered at Day 9, 10 or 11 after insemination.

The insemination conditions did not affect pregnancy rate, percentage embryonic mortality and average embryonic development. Socially restricted housing conditions caused a reduced reproductive performance (puberty attainment, pregnancy rate) (Chapter 2).

The behaviour of the gilts during the contact period with the vasectomized boar after insemination is described in Chapter 3. Gilts of the socially restricted conditions showed a different introductory sexual behaviour; the majority of the socially restricted sows did not show an immediate standing response upon introduction of the boar, but initiated contact with the boar. Once the standing response was elicited, mating behaviour was similar in the two groups of gilts.

Effects of insemination conditions and social conditions on within-litter embryonic diversity are described in Chapter 4. The stimulus-rich insemination caused a decrease in within-litter embryonic diversity in the socially non-restricted gilts. In contrast, in gilts from the socially restricted conditions, the stimulus-rich insemination caused an increase in within-litter embryonic diversity. Accepting the hypothesis that a low rate of within-litter diversity is related to less embryonic mortality, these results correspond with the differences in litter size as reported in literature; boar stimulation results in an increase in litter size in socially non-restricted sows and in a decrease in litter size in socially restricted sows. This effect possibly is caused by the stressful conditions of the socially restricted gilts which also leads to a lower reproductive performance (Chapter 2) and a changed behaviour towards the boar (Chapter 3).

Effects as found in Experiment I were verified in a second experiment with similar insemination conditions. Additionally, effects of boar stimulation were studied during an earlier stage of pregnancy. The experiment involved gilts from socially restricted conditions only (Chapter 5). The gilts were slaughtered at Day 5/6 or Day 11 after insemination. In contrast to Experiment I, a stimulus-rich insemination did not influence within-litter embryonic diversity, but influenced the duration of oestrus, embryonic development and the number of accessory sperm cells (sperm cells bound to the zona pellucida of the embryos). The latter indicates an

influence of boar stimulation on both ovulation and sperm transport. Insemination conditions did not affect reproductive performance (pregnancy rate, embryonic mortality rate), which was better than in Experiment I.

From these two experiments, it became clear that boar stimuli around insemination may affect reproductive parameters during early pregnancy, including early within-litter embryonic diversity. Moreover, seemingly similar conditions may give rise to different results. Based on these findings, it was concluded that studies investigating influences of boar stimuli around insemination should focus on more direct effects of these stimuli. Especially the process of ovulation seems of interest, because the duration of ovulation (interval between the ovulation of the first and the last oocyte during oestrus) may be influenced by boar stimuli (Chapter 1, Chapter 5) and because the duration of ovulation has been suggested to be an important cause of embryonic diversity.

Assessment of the duration of ovulation and its relation with embryonic diversity

The second part of this study was aimed at the development and use of transrectal ultrasonography as first nonsurgical method to assess the moment and duration of ovulation in individuals and link it to embryonic development and embryonic diversity (Chapter 6 - 7).

Up to now, estimates of the duration of ovulation in individual sows were based on the use of repeated laparoscopy. It is not clear if and to what extent the duration of ovulation is influenced by the lengthy anaesthesia associated with repeated laparoscopy. In the present study, transrectal ultrasonography to assess the duration of ovulation was developed and applied.

In order to assess the duration of ovulation per sow, it is necessary to determine the number of follicles on both ovaries accurately and repeatedly. The use and accuracy of transrectal ultrasonography to assess the number of follicles is evaluated in Chapter 6. The deviation between the maximum follicle count and the corpora lutea count at 100 hours after ovulation was zero or only one in 80% (21 of 26) of the ovaries. This deviation was not related to the corpora lutea count. To assess the duration of ovulation, the number of follicles on both ovaries was counted at 30-minute intervals. The process of ovulation was observed as a consistent decrease in the number of follicles over time. The duration of ovulation varied between sows (1 to 7 hours) and between groups of sows (1.8 ± 0.6 to 4.6 ± 1.7 hours). These groups differed in many respects, e.g. housing conditions, induction of ovulation, insemination treatment. At this moment, it is unknown which factor caused this variability in the duration of ovulation.

To investigate whether the frequent use of transrectal ultrasonography affected reproductive parameters, scanned and non-scanned sows were slaughtered at 160 hours after ovulation-induction (Chapter 7). Neither the process of ovulation (indirectly judged by embryonic development and embryonic diversity) nor sperm transport (indirectly judged by fertilization rate and number of accessory sperm cells) seemed affected by frequent transrectal ultrasonography.

In two groups of sows, in which the duration of ovulation varied between 1 and 4 hours, no relationship was found between the duration of ovulation and early embryonic diversity, neither at Day 4 after ovulation (Chapter 6) nor at Day 5 after ovulation (Chapter 7) (the group of sows with a duration of ovulation beyond 4 hours was not inseminated). It was concluded that the duration of ovulation is not an important cause of differences in embryonic diversity between sows and hence presumably is not an important determinant for embryonic mortality.

In the General Discussion, possible causes of differences in embryonic diversity between litters are summarized. Based on results from Chapter 6 and Chapter 7, it seems likely that, even under seemingly optimal insemination conditions a lengthened duration of fertilization (interval between the fertilization of the first and the last oocyte during oestrus) may exist, causing an increase in embryonic diversity.

The method of transrectal ultrasonography is an indispensable tool in studies in which the moment of ovulation needs to be assessed, for example in studies concerning insemination strategies.

Conclusions

Boar stimuli immediately after insemination affect the duration of oestrus, the number of accessory sperm cells, embryonic development and embryonic diversity. This suggests that boar stimuli affect the process of ovulation and the transport of sperm. By unknown causes, the influence of boar stimuli varies between experiments.

Effects of boar stimuli around insemination on litter size, as reported in literature, seem to originate from differences in within-litter embryonic diversity and associated changes in embryonic mortality.

Transrectal ultrasonography is a good method to count the number of follicles repeatedly and accurately and to monitor the moment and duration of ovulation. There are no indications that the process of ovulation and sperm transport are influenced by the procedure.

The duration of ovulation, as assessed by transrectal ultrasonography, varied between sows (from 1 to 7 hours) and varied between groups of sows (from 1.8 ± 0.6 to 4.6 ± 1.7 hours). The cause(s) of this variability is (are) unknown.

The duration of ovulation (varying between 1 and 4 hours) was not related with embryonic diversity. Consequently, the duration of ovulation may neither be an important determinant for embryonic diversity, nor for associated embryonic mortality.

Differences in within-litter embryonic diversity are related to the number of accessory sperm cells (sperm cells bound to the zona pellucida of the embryos). This suggests that the duration of fertilization is an important determinant for embryonic diversity and associated embryonic mortality.

SAMENVATTING

SAMENVATTING

Inleiding

In de varkenshouderij wordt verondersteld dat het gebruik van ofwel kunstmatige inseminatie (KI) ofwel natuurlijke dekking (ND) leidt tot vergelijkbare reproductieresultaten. Mogelijke verschillen in reproductieresultaten tussen KI en ND zijn echter vrijwel niet experimenteel onderzocht. Verder blijkt uit experimenteel onderzoek (literatuuroverzicht, Hoofdstuk 1) dat specifieke beerstimuli wel invloed hebben op reproductieprocessen zoals stareflex, ovulatieproces en spermatransport. Deze kunnen vervolgens leiden tot verschillen in drachtigheidspercentage en worpgrootte. Zowel uitwendige stimuli (met name olfactorische en tactiele) als inwendige stimuli (met name componenten van spermaplasma) zijn hierbij van belang. Ook blijkt uit dit overzicht (Hoofdstuk 1) dat effecten van beerstimuli ten aanzien van worpgrootte en drachtigheidspercentage ondermeer afhankelijk zijn van de sociale condities van de zeugen. Het is mogelijk dat hierdoor een verschil in reproductieresultaten tussen KI en ND gemaskeerd wordt.

De verschillen in worpgrootte zoals die onder experimenteel gevarieerde inseminatiecondities gevonden worden, kunnen een gevolg zijn van bevruchtingsproblemen. Ze zijn echter zeer waarschijnlijk een gevolg van verschillen in embryonale sterfte. Worpgrootte bij varkens wordt namelijk met name bepaald door het niveau van embryonale sterfte. Alhoewel dit laatste reeds lang algemeen geaccepteerd is, bestaat ten aanzien van de oorzaken van embryonale sterfte nog veel onduidelijkheid. Recentelijk (80-er jaren) werden aanwijzingen verkregen dat variatie in embryonale ontwikkeling binnen tomen een belangrijke oorzaak is van embryonale sterfte die optreedt na Dag 11 van de dracht. Rondom Dag 11 beginnen de verst ontwikkelde embryo's met de productie van oestrogenen, die vervolgens het uteriene milieu wijzigen. Dit gewijzigde uteriene milieu stimuleert vervolgens de ontwikkeling van deze embryo's. Echter, een deel van de minder ver ontwikkelde embryo's zal niet in staat zijn om zich in dit veranderde milieu verder te ontwikkelen en sterven af.

Effect van inseminatiecondities op de vroege dracht

Op grond van het bovenstaande werd de hypothese getoetst of inseminatiecondities effect hebben op reproductieparameters gedurende de vroege dracht, met name op binnen-toom variatie in vroeg embryonale ontwikkeling. Het eerste deel van het beschreven onderzoek is daarom gericht op de bestudering van de invloed van beerstimuli rondom inseminatie op binnen-toom

variatie in embryonale ontwikkeling en op andere aspecten van de vroege dracht (zoals het embryonale sterftepercentage, het drachtigheidspercentage en de bronstduur) (Hoofdstuk 2 - 5).

In een eerste experiment werden de inseminatiecondities gevarieerd bij gelten die vanaf een leeftijd van ca. 5 maanden onder verschillende sociale condities werden gehuisvest: ofwel sociaal-arm (individueel gehuisvest zonder beercontact) ofwel sociaal-rijk (paarsgewijs gehuisvest + één maal daags beercontact vanaf een leeftijd van circa 6 maanden). De inseminatie, die bij de derde bronst na aanvang van de puberteit plaatsvond, werd ofwel stimulus-rijk (KI + 20 minuten contact met een gevasectomeerde beer) ofwel stimulus-arm (KI) uitgevoerd. Het gebruik van KI gaf de mogelijkheid om standaardcondities te creëren ten aanzien van het aantal geïnsemineerde spermacellen en het genotype van de beer. De stimuluswaarde van een gevasectomeerde beer is grotendeels vergelijkbaar met die van een intacte beer (zie Hoofdstuk 1). De gelten werden vervolgens 9, 10 of 11 dagen na inseminatie geslacht.

Beide inseminatiecondities leverden geen verschillen op in drachtigheidspercentage, percentage embryonale sterfte en gemiddelde embryonale ontwikkeling. De sociaal-beperkte huisvestingsomstandigheden bleken een negatief effect te hebben op de reproductieresultaten (puberteit, drachtigheidspercentage) (Hoofdstuk 2).

Het gedrag van de gelten tijdens het contact met de gevasectomeerde beer na de inseminatie, wordt beschreven in Hoofdstuk 3. Gelten uit de sociaal-arme condities vertoonden een afwijkende introductie tot het sexuele gedrag; het merendeel van deze gelten vertoonde niet onmiddellijk de sta-reflex, maar initieerde contact met de beer. Vanaf het moment dat de sta-reflex optrad, reageerden beide groepen gelten vergelijkbaar.

Effecten van de sociale condities en de inseminatiecondities op de binnen-toom variatie zijn beschreven in Hoofdstuk 4. De stimulus-rijke inseminatie resulteerde bij gelten uit de sociaal-rijke condities in een verlaging van de binnen-toom variatie. Echter, bij gelten uit de sociaal-arme condities, leidde dit tot een verhoging van de binnen-toom variatie. Er van uitgaande dat een lagere binnen-toom variatie is gerelateerd aan een lagere embryonale sterfte, komen de resultaten overeen met verschillen ten aanzien van worpgrootte, zoals deze in de literatuur zijn gerapporteerd. Beerstimuli hebben grotere worpen tot gevolg in sociaal-rijk gehuisveste zeugen en kleinere worpen in sociaal-arm gehuisveste zeugen. Dit effect zou een gevolg kunnen zijn van de stressrijke omstandigheden in de sociaal-arme huisvesting, die ook leiden tot verslechterde reproductieresultaten (Hoofdstuk 2) en een afwijkend gedrag ten opzichte van de beer (Hoofdstuk 3).

De in Experiment I gevonden effecten van beerstimulatie werden geverifieerd in een tweede experiment met vergelijkbare inseminatiecondities. Tevens werden de effecten bestudeerd tijdens een vroegere fase van de dracht. Dit experiment betrof alleen sociaal-beperkt gehuisveste

gelten (Hoofdstuk 5). De gelten werden geslacht op Dag 5/6 of Dag 11 na inseminatie. In tegenstelling tot Experiment I, leidde een stimulus-rijke inseminatie niet tot beïnvloeding van binnen-toom variatie in embryonale ontwikkeling maar beïnvloedde wel de bronstduur, de embryonale ontwikkeling en het aantal accessoire spermacellen (spermacellen gebonden aan de zona pellucida van de embryo's). Dit laatste geeft aan dat waarschijnlijk zowel het ovulatieproces als het spermatransport beïnvloed werden. De verschillende inseminatieomstandigheden leverden ook in het tweede experiment geen verschillen op in de reproductieresultaten (drachtigheidspercentage, percentage embryonale sterfte), welke beter waren dan in het eerste experiment.

Uit deze twee experimenten werd duidelijk dat beerstimuli rondom inseminatie in staat zijn om meerdere reproductieparameters tijdens de vroege dracht, inclusief binnen-toom variatie, te beïnvloeden. Bovendien bleek dat ogenschijnlijk vergelijkbare condities tot zeer verschillende resultaten aanleiding kunnen geven. Op grond hiervan werd geconcludeerd dat studies naar invloeden van beerstimuli zich moeten richten op meer directe effecten van beerstimuli. Met name het ovulatieproces lijkt hierbij van belang, ondermeer omdat de ovulatieduur (periode tussen de ovulatie van de eerste en de laatste eicel tijdens een bronst) door beerstimuli beïnvloedbaar lijkt (Hoofdstuk 1, Hoofdstuk 5) en omdat ovulatieduur wordt beschouwd als een belangrijke oorzaak voor de gevonden binnen-toom variatie.

Bepaling van de ovulatieduur en de relatie van de ovulatieduur met binnen-toom variatie

Het tweede deel van het beschreven onderzoek was gericht op de ontwikkeling en het gebruik van transrectale echografie als eerste niet-chirurgische methode om het ovulatiemoment en de ovulatieduur te bepalen en deze vervolgens te koppelen aan (variatie in) embryonale ontwikkeling (Hoofdstuk 6 - 7).

Tot nu toe waren schattingen van de ovulatieduur bij individuele zeugen gebaseerd op het gebruik van herhaalde laparoscopie rondom ovulatie, wat betekent dat de zeugen langdurig onder narcose waren. Het is onduidelijk of en in hoeverre het ovulatieproces hierdoor wordt beïnvloed. In de huidige studie is daarom het gebruik van transrectale echografie ter bestudering van het ovulatieproces ontwikkeld en toegepast.

Om de ovulatieduur bij individuele zeugen te bepalen is het noodzakelijk om het aantal follikels op beide ovaria nauwkeurig en frequent te bepalen. In Hoofdstuk 6 wordt het gebruik en de nauwkeurigheid van transrectale echografie ten aanzien van de follikeltellingen

geëvalueerd; bij 80% van de ovaria (21/26) was de afwijking tussen de maximale follikeltelling en de corpora lutea telling op circa 100 uur na ovulatie hooguit één. De afwijking was niet gerelateerd aan het aantal corpora lutea. Ter bepaling van de ovulatieduur werd het aantal follikels op beide ovaria elk half uur geteld. Het ovulatieproces is waarneembaar als een consistente afname van het aantal follikels in de tijd. De ovulatieduur varieerde tussen zeugen (van 1 tot 7 uur) en tussen groepen zeugen (van 1.8 ± 0.6 tot 4.6 ± 1.7 uur). Deze groepen verschilden ondermeer ten aanzien van de huisvestingscondities, ovulatieinductie en inseminatiecondities. De oorzaak van deze variatie is vooralsnog onduidelijk.

In Hoofdstuk 7 werd gecontroleerd of het herhaald gebruik van transrectale echografie effect heeft op reproductieparameters, bepaald op 160 uur na ovulatie-inductie. Noch het ovulatieproces (indirect beoordeeld aan de hand van embryonale ontwikkeling en variatie in embryonale ontwikkeling), noch het spermatransport (indirect beoordeeld aan de hand van bevruchtingspercentage en aantal accessoire spermacellen) leken beïnvloed door herhaalde echografie.

In twee groepen zeugen, waarin de ovulatieduur varieerde tussen 1 en 4 uur, werd geen relatie gevonden met binnen-toom variatie in embryonale ontwikkeling, noch op dag 4 na ovulatie (Hoofdstuk 6), noch op dag 5 na ovulatie (Hoofdstuk 7) (zeugen uit de proefgroep waarin de ovulatieduur langer dan 4 uur was, waren niet geïnsemineerd). Hieruit werd geconcludeerd dat de ovulatieduur geen belangrijke oorzaak is van tussen zeugen bestaande verschillen in binnen-toom variatie in embryonale ontwikkeling. Daardoor is het waarschijnlijk ook geen belangrijke voorspeller van embryonale sterfte.

In de Algemene Discussie worden ondermeer mogelijke oorzaken van verschillen in binnen-toom variatie tussen tomen aangegeven. Op grond van resultaten uit Hoofdstuk 6 en Hoofdstuk 7 lijkt het aannemelijk dat zelfs onder ogenschijnlijk optimale inseminatieomstandigheden extra binnen-toom variatie kan ontstaan als gevolg van een verlengde fertilisatieduur (periode tussen de fertilisatie van de eerste en de laatste eicel tijdens een bronst).

De ontwikkelde methode van transrectale echografie is een onmisbare hulp in studies waarin het ovulatiemoment bepaald dient te worden, zoals in studies naar inseminatiestrategieën.

Conclusies

Beerstimuli onmiddellijk na inseminatie hebben effect op bronstduur, aantal accessoire spermacellen, embryonale ontwikkeling en variatie in embryonale ontwikkeling, wat aangeeft dat ze waarschijnlijk zowel het ovulatieproces als het spermatransport beïnvloeden. Door nog onbekende oorzaken varieert hun invloed tussen experimenten.

In de literatuur gerapporteerde invloeden van beerstimuli rondom inseminatie op worpgrootte, lijken te verlopen via effecten op binnen-toom variatie en de daarmee samenhangende embryonale sterfte.

Transrectale echografie is een goede methode om het aantal follikels herhaaldelijk en nauwkeurig te bepalen en het ovulatiemoment en de ovulatieduur te observeren. Er zijn geen aanwijzingen dat het ovulatieproces of het spermatransport worden beïnvloed door de procedure.

De ovulatieduur zoals bepaald met behulp van transrectale echografie varieerde tussen zeugen (van 1 tot 7 uur) en varieerde tussen groepen zeugen (van 1.8 ± 0.6 tot 4.6 ± 1.7 uur). De oorzaak van deze variatie is onbekend.

De ovulatieduur (variërend tussen 1 en 4 uur) was niet gerelateerd aan verschillen in binnen-toom variatie in embryonale ontwikkeling. Daarom lijkt de ovulatieduur geen belangrijke determinant van binnen-toom variatie, noch van de daarmee samenhangende embryonale sterfte.

Verschillen in binnen-toom variatie in embryonale ontwikkeling zijn gerelateerd aan het gemiddeld aantal accessoire spermacellen. Dit suggereert dat de fertilisatieduur een belangrijke determinant is van binnen-toom variatie en van de daarmee samenhangende embryonale sterfte.

CURRICULUM VITAE

Nicoline Margaretha Soede werd op 9 maart 1964 geboren in Zegveld (ZH). In 1972 verhuisde zij naar Overschild (Gr). In 1982 behaalde zij in juni het Atheneum diploma aan de Christelijke scholengemeenschap 'het Fivelcollege' te Delfzijl en begon in september met de studie Zoötechniek aan de toenmalige Landbouwhogeschool te Wageningen. In juni 1987 sloot zij de studie Zoötechniek af, met als afstudeervakken Gezondheids- en Ziekteleer, Vruchtbaarheid en Voortplanting en Agrarische Bedrijfs Economie.

Na haar afstuderen was zij tot en met februari 1988 werkzaam als toegevoegd onderzoekster bij de sectie Ontwikkelingsbiologie van de vakgroep Experimentele Diermorfologie en Celbiologie van de Landbouwuniversiteit te Wageningen. Per 1 maart volgde de aanstelling als Assistent in Opleiding bij de toenmalige sectie Gezondheids- en Ziekteleer van de vakgroep Veehouderij voor het onderzoek dat resulteerde in dit proefschrift. Vanaf 1 mei 1992 is zij werkzaam als universitair docent bij de sectie Gezondheidsleer en Reproductie van de vakgroep Veehouderij.