Optimising insemination strategies in pigs

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Optimising insemination strategies in pigs

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Timing of insemination is one of the factors that influence reproduction efficiency because of its direct effect on fertilisation. The research described in this thesis dealt with possibilities to optimise insemination strategies at farms. From experiments it could be concluded that fertilisation results were not very sensitive to variation in the number of inseminated sperm cells in the range of 1×10^9 to 6×10^9 sperm cells. However a combination of suboptimal circumstances like a low sperm dosage and substantial loss of sperm cells due to backflow during insemination resulted in sub-optimal fertilisation. A mathematical model was described of chances on fertilisation in relation to the interval between insemination and ovulation in sows. A PIG Simulation model for Insemination Strategies (PIGSIS) was developed. Many physiological processes are included in PIGSIS e.g. fertilisation, embryonic mortality (due to: degeneration, maternal recognition of pregnancy and embryonic uterine capacity) and foetal mortality (due to foetal uterine capacity). Optimal timing of insemination in PIGSIS is based on specific farm parameters like oestrus duration and the relation between weaning to oestrus interval and oestrus duration since oestrus duration is a reasonable estimate for ovulation. PIGSIS can be used to study reproduction results in relation to insemination strategies on farms.

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STELLINGEN

1. Voor een goede inseminatiestrategie op bedrijfsniveau moet bij het varken naast het begin ook het eind van de bronst worden bepaald. *(dit proefschrift)*

2. Inseminatiestrategieën bij varkens moeten aan de bedrijfssituatie worden aangepast en niet andersom. *(dit proefschrift)*

3. Aangezien met 1 miljard spermacellen vergelijkbare bevruchtingsresultaten kunnen worden gehaald als met 3 miljard spermacellen *(dit proefschrift)* zouden varkens-KI organisaties een diversiteit in dosisgrootte aan kunnen bieden afhankelijk van de wensen van een bedrijf.

4. Terugvloei van sperma na inseminatie heeft onder normale omstandigheden geen invloed op de bevruchtingsresultaten bij varkens. *(dit proefschrift)*

5. Simulatiemodellen zijn een goed middel om de kennis van procesonderdelen, voortgekomen uit afzonderlijke experimenten, te koppelen en daardoor inzicht te vergroten in het gehele proces.

6. In het onderzoek naar embryonale sterfte wordt het effect van bevruchting meestal niet onderkend.

7. De onzekerheid over het toekomstige beleid van de overheid is een factor die meer stagnerend is voor bedrijfsontwikkeling in de agrarische sector dan de lage opbrengstprijzen.

8. Door het instellen van een mestquotum voor burgers zou er veel begrip voor mestproblematiek in de agrarische sector kunnen ontstaan.

9. De voortdurende reorganisaties van de Wageningen Universiteit (Landbouwhogeschool, Landbouwuniversiteit Wageningen) laat weinig ruimte voor een evaluatie, zodat de kwaliteit van de veranderingen nauwelijks beoordeeld wordt.

10. De wereld zou er waarschijnlijk anders hebben uitgezien als God zijn dochter zou hebben gestuurd in plaats van zijn zoon.

11. Een goede inseminatiestrategie dekt de taak van een beer.

12. Het leven is een groot feest maar je moet wel zelf de slingers ophangen.

Stellingen behorend bij het proefschrift:
"Optimising insemination strategies in pigs",
D.W.B. Steverink,
Wageningen, 22 oktober 1999

Aan mijn ouders

Voorwoord

Op de middelbare school fantaseerden klasgenoten over de boeken die ze later gingen schrijven. Voor mij was in die tijd een ding duidelijk dat ik daar nooit over zou dromen. Maar zie hier nu heb ik toch een boek geschreven en ben ik wellicht een van de weinigen uit die klas die dat heeft gedaan.

Tijdens dit vier jarig onderzoek hebben veel mensen mij gesteund en geholpen en deze wil ik graag bedanken. Op de eerste plaats waren dat Bas Kemp en Nicoline Soede. Jullie waren voor mij het 'duo' van steun. De een voor de grote lijnen en de ander voor de kritische blik op de details. Het pad leidend tot eenstemmigheid ging soms over hobbels van spraakversnelling en stemverheffing, maar op het eind werd de lucht altijd geklaard door gezellig een shaggie op te steken. Nicoline en Bas bedankt voor die vele uren van begeleiding. Beste Jos, promotor, in de eerste twee jaar was de begeleiding intensiever dan de laatste twee jaar doordat je naar Utrecht bent gegaan. De PTT werkte ook nog eens niet mee om de stukken heen en weer te krijgen. Desondanks heb ik veel steun van je nuchtere 'niet-repro' kijk op mijn manuscripten gehad. Ook wil ik Wiebe Koops bedanken voor de intensieve ondersteuning in het laatste jaar. Vele keren heb ik aan je bureau gestaan om iets over mathematische modellen te vragen en altijd wist je een goede oplossing voor mijn problemen.

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Collega's op je werk zijn één van de belangrijkste factoren die een dag wel of niet kunnen laten slagen. Ik moet zeggen dat ze bijna altijd slaagden bij Veehouderij. Gezellige club, niet in de laatste plaats bij de bitterballen- en frikadellenparty's. De AIO-zolder is ook een van de opmerkelijkste hokken waar mensen werken op Zodiac, zeker

nu er een airco (Jos, ook daar voor hartelijk dank) staat. Henry, John, Gustavo, Pieter en vele anderen bedankt voor de gezelligheid op de AIO-zolder en bij Veehouderij. Pieter, ik vind het dan ook fijn dat je als een van mijn collega's mij wilt ondersteunen als paranimf tijdens mijn promotie.

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Dorothé

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1 General introduction

1 General introduction

INTRODUCTION

Reproductive efficiency in pigs can be defined as the total number of piglets born per sow per year at farms which has large variation between farms (Clark and Leman, 1987; Stein et al., 1990; Dewey et al., 1995). Various factors are involved in the reproduction efficiency such as: housing system (O'Grady et al., 1983), season (Koketsu et al., 1997; Xue et al., 1994 d406), parity (Xue et al., 1994), oestrus detection strategy (Dewey et al., 1995), lactation length (Clark and Leman, 1987) and weaning to oestrus interval (Vesseur et al., 1994). Reproduction efficiency can be seen as an accumulation of many physiological processes (Figure 1). One of the first physiological processes in this accumulation is fertilisation of the ovulated oocytes.

Besides fertilisation, many events occur during a reproduction cycle. A few days after weaning (on average 5 to 6 days) sows start to show oestrus (Figure 1).

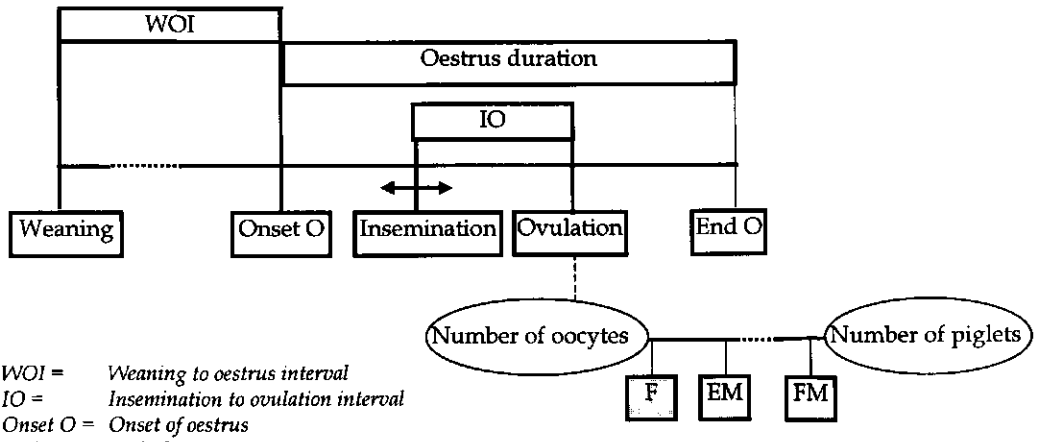

Figure 1. Time scale of reproduction events starting at weaning and ending in the number of piglets at farrowing of sows.

During the oestrus period, either natural mating or artificial insemination can be performed. At insemination or mating, billions of sperm cells are deposited at the utero-cervical junction. Due to uterine contractions, sperm cells migrate through the uterine horns (1.5 m) to the oviducts (Einarsson, 1985). A relatively small number (about 0.01%) of sperm cells reach the oviduct where they are temporarily stored in the sperm reservoir and are protected against reduction in motility, in viability and in the capacity to fertilise (Overstreet et al., 1980; Suarez et al., 1991). At ovulation the remained sperm cells are released from the sperm reservoir and move to the ampullary-isthmic junction where fertilisation takes place if the oocytes and sperm cells are still capable to fertilise. The fertile lifespan of oocytes and sperm cells, border the period in which insemination can lead to successful fertilisation (Hunter, 1995). This is confirmed by a study of Soede et al. (1995a) in which it was concluded that there was an optimal period for insemination in relation to the moment of ovulation. The best fertilisation results were found when insemination was performed between 24 and 0 h before ovulation. In their study a normal commercial sperm dosage was used with 3×10^9 sperm cells. Similar results were found in Germany and Denmark (Waberski et al., 1994; Nissen et al., 1997). Waberski et al. (1994) defined an optimal insemination time between 12 and 0 h before ovulation in gilts. Nissen et al. (1997) found the highest reproduction results in terms of number of day 10 embryos, of farrowing rate and of litter size in sows that were inseminated between 28 h before ovulation and 4 h after ovulation. From these studies it can be concluded that the time interval between insemination and ovulation is related to the fertilisation results and reproduction results. Good insemination results will only be obtained within a restricted time interval from ovulation. Therefore, it will be important to predict the moment of ovulation for optimising fertilisation and defining a good insemination strategy.

Some potential predictors for ovulation have been studied such as: onset of oestrus (Table 1), vaginal mucus conductivity (Stokhof et al., 1996), vaginal temperature (Soede et al., 1997) and follicle diameter (Nissen et al., 1995). Unfortunately, these parameters did not properly predict the moment of ovulation. The preovulatory LH surge could be a good predictor (Soede et al., 1994) but current methods are not suitable for practical application on commercial farms. From studies shown in Table 1, it is concluded that ovulation takes place at a rather fixed moment during oestrus. The average relative moment of ovulation varied between 67% and

72% of oestrus. This means that the oestrus duration could be a good estimator of ovulation but unfortunately this estimator is a retrospective one.

Table 1. Mean ± SD and range of the oestrus duration, relative ovulation during oestrus and the moment of ovulation after onset of oestrus.

Oestrus duration (h)		Relative ovulation (%)		Ovulation after onset of oestrus (h)			
Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	N	Reference[a]
60 ± 15	32-96	71 ± nd[b]	35-100	45 ± 13	15-85	483	1
56 ± 8	46-73	68 ± 8	54-78	37 ± 2	35-43	20	2
50 ± 13	24-88	72 ± 15	39-133	35 ± 8	10-58	144	3[c]
60 ± 11	32-88	67 ± 8	42-94	41 ± 8	22-58	91	4
60 ± 14	30-89	71 ± 14	38-118	nd	17-70	91	5

[a]1: Weitze et al. (1994); 2: Mburu et al. (1995); 3: Soede et al. (1995a); 4: Soede et al. (1995b); 5: Nissen et al. (1997);

[b] nd = not determined;

[c] Sows with a weaning to oestrus interval of more than 8 days were excluded (n=2).

Oestrus duration shows variation between experiments and also within experiments (Table 1). One of the factors affecting oestrus duration is the interval of weaning to onset of oestrus. In a study of Kemp and Soede (1996) the oestrus duration decreased from 61 to 53, to 49 and to 38 h when onset of oestrus occurred at day 3 to day 4, 5 and 6 after weaning, respectively. Similar negative relations between oestrus duration and weaning to oestrus interval were found by Weitze et al. (1994) and Nissen et al., (1995). When more factors, that are related to oestrus duration, become available, oestrus duration might be predictable from which ovulation can be estimated.

STUDY OBJECTIVE

From the foregoing it can be concluded that there is a lack of knowledge with respect to the improvement of insemination strategies in pigs. The research described in this thesis deals with the possibility of developing a method to optimise insemination strategies on individual farms. This method should aid to a better understanding of the reproduction process and the effects of timing of insemination. To reach this goal different physiological processes like oestrus, ovulation,

embryonic and foetal mortality will be combined. An optimal insemination strategy in this thesis is defined as: a maximal fertilisation with an efficient use of semen and a low number of inseminations. There are two important issues to consider in the development of an optimal insemination strategies (1) the effect of the moment of insemination in relation to ovulation on fertilisation results, and (2) possibilities to predict the moment of ovulation.

For this thesis three objectives are formulated:

1. Increase insight in the effects of the interval between insemination and ovulation on fertilisation results.
2. Increase knowledge on the possibilities of predicting the moment of ovulation of sows at farm level.
3. Develop a method which can be used for optimising insemination strategies at commercial farms.

The variety of factors influencing the reproduction makes a model and simulation approach of interest. A mathematical model might be helpful since underlying processes can be controlled.

OUTLINE OF THE THESIS

This study consists of 3 parts presented as Chapter 2, 3 and 4 in this thesis. In Chapter 2 the relation of the interval between insemination and ovulation and fertilisation was studied. The sensitivity of this relation is studied by using different semen dosages (Chapter 2.1) and by investigating the effect of the amount of semen backflow (Chapter 2.2). The knowledge about fertilisation in relation to insemination to ovulation interval is summarised in Chapter 2.3, in which a mathematical model for conception and fertilisation is presented.

In Chapter 3, differences in oestrus duration at farm level are studied. The consistency of the average oestrus duration of farms is studied and to what extent this information could be used in a prospective way to predict oestrus duration on farms. The effects of insemination strategies, as applied on these farms, on reproduction results are investigated as well (Chapter 3.1).

Chapter 4 describes the development of the PIG Simulation model for Insemination Strategies (PIGSIS) (Chapter 4.1). Information of Chapter 2 and 3 together with literature are used for estimating parameters in PIGSIS. Sensitivity analyses and validation are carried out to test the accuracy of PIGSIS.

Finally, the results of the three chapters are discussed in the General discussion (Chapter 5).

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2 Fertilisation in relation to insemination and ovulation

2.1 Sperm cell dosage

Influence of insemination to ovulation interval and sperm cell dosage on fertilisation in sows

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Influence of insemination to ovulation interval and sperm cell dosage on fertilisation in sows

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ABSTRACT

This experiment was conducted to determine effects of sperm dosage at insemination on fertilisation rates and accessory sperm cells attached to day 5 embryos. Multiparous sows (n=115) were artificially inseminated once with 1, 3 or 6 $\times$ 10^9 sperm cells between 3 to 48 h before ovulation. Transrectal ultrasonography was performed at intervals of 4 h to determine the time of ovulation and sows were killed at 120 $\pm$ 5 h after ovulation to assess results of fertilisation. The insemination to ovulation interval is of major influence on the fertilisation rate and accessory sperm count. A nonsignificant but consistent increase in fertilisation rate and in number of accessory sperm cells due to sperm dosage was observed. In the insemination to ovulation interval of 12-24 h the median fertilisation rates were 95%, 100% and 100%, and the median accessory sperm count was 11, 17 and 31 for the dosages 1, 3 and 6 $\times$ 10^9 dosages, respectively. In the insemination to ovulation interval of 24-36 h the median fertilisation rate was 88, 95 and 97% and the median accessory sperm count was 6, 8 and 11 for the dosages 1, 3 and 6 $\times$ 10^9 dosages, respectively. No direct relationship was detected between embryo quality and the accessory sperm count but there was a relationship between insemination to ovulation interval and accessory sperm count. The fertilisation rate was positively correlated to the breeding value for litter size of the sows. In conclusion, the effects of sperm dosage on fertilisation rate and on accessory sperm count in sows were small and non significant, indicating only small effects of sperm dosage on functioning of the sperm reservoir in the sow.

INTRODUCTION

At insemination or mating in pigs, billions of sperm cells are deposited in the uterus. From this site, spermatozoa start to migrate towards the site of sperm storage which is in the first 2 cm of the caudal region of the isthmus in the oviduct (Hunter, 1981). When sperm cells reach the reservoir, they can be stored without a reduction of the motility, viability and fertilisation capacity (Overstreet et al., 1980; Suarez et al., 1991), which makes the reservoir a temporal shelter to bridge the time until fertilisation of oocytes. Sperm cells can be stored in the reservoir for up to 40 h (Hunter, 1981; Pollard et al., 1991; Raycoudhurry and Suarez, 1991).

Relative to the inseminated number of sperm cells, only a small number reaches the isthmic sperm reservoir (Hunter, 1981). Before the sperm cells reach the oviduct they have to pass a major barrier: the uterotubal junction (UTJ) (Smith et al., 1987). Dead (Viring, 1980), heterologous (pigs: Baker et al., 1968; hamster: Smith et al., 1988) or capacitated spermatozoa (Shalgi et al., 1992) pass the UTJ not as good as normal spermatozoa. Spermatozoa that do not reach the sperm reservoir in time are killed by the hostile uterine environment. In the hamster uterus, the motility of spermatozoa decreases from 60% immediately before insemination to 10% at 1 h after insemination (Smith et al., 1988). The spermatozoa that do not reach the sperm reservoir, are removed by backflow (Viring and Einarsson, 1981) or local phagocytosis which is seen within 2 h after insemination (Pursel et al., 1978).

Fertilisation rate is dependent on the time interval between insemination and ovulation. In sows, it was shown that fertilisation rate is optimal when insemination with 3×10^9 sperm cells occurs between 0 and 24 h before ovulation (Soede et al.,1995a). Nissen et al. (1996) found an optimal insemination time, with 2×10^9 sperm cells, between 28 h before ovulation and 4 h after ovulation, based on a high farrowing rate and a large litter size in multiparous sows. Furthermore, Soede et al. (1995a) showed that the number of accessory sperm cells attached to the zona pellucida of the embryos fell rapidly when the interval between insemination and ovulation increased. Accessory sperm represent a population of spermatozoa able to traverse the barriers of the female reproductive tract and partially penetrate the zona pellucida at fertilisation (Weitze et al., 1988; Saacke et al., 1994). With an increase of the interval between insemination and ovulation, the number of potentially fertilising sperm cells and, consequently, the accessory sperm cells and also the fertilisation rate decreased. The

question arises whether it is possible to increase the number of sperm cells at the site of fertilisation by increasing the number of sperm cells in the reservoir by using a greater number of sperm cells at insemination. This might extend the insemination to ovulation interval in which good fertilisation results can be achieved compared with the 0-24 h found with 3×10^9 sperm cells. A second question is whether the number of sperm cells at the site of fertilisation might be decreased by decreasing the number of sperm cells at insemination and, thereby, shortening the insemination to ovulation interval in which good fertilisation results can be achieved compared to the 0-24 h found with 3×10^9 sperm cells. In an in vitro study (Lefebvre and Suarez, 1996), the number of spermatozoa bound to oviductal epithelium was found to be dependent on the concentration of sperm cells. Bovine isthmus epithelium was incubated for 15-30 minutes with 1×10^5 motile sperm cells, after which 30 sperm cells bound to 0.1 mm^2 epithelium, whereas incubation with 1×10^6 sperm cells resulted in 600 sperm cells bound to 0.1 mm^2 epithelium. The change in number of sperm cells bound to oviduct epithelium in vitro might also occur in vivo and, as a consequence, increase fertilisation rate.

The two hypotheses of this study are (1) that fertilisation rates are lower when a lower number of sperm cells (1×10^9) is inseminated within 24 h of ovulation; and (2) that fertilisation rates are higher when a greater number of sperm cells (6×10^9) is inseminated more than 24 h from ovulation. The objective of this study is to investigate the effects of the number of sperm cells at insemination on fertilisation rate and accessory sperm cells at 5 days after ovulation.

MATERIALS AND METHODS

Animals and housing

For a period of 16 weeks, every 2 weeks, 9 to 22 sows (139 in total) were obtained at the day of weaning. At the experimental farm, the sows were housed individually in crates and received a total of 2.5 kg of a commercial sow diet (12.9 MJ ME kg^{-1}) in two portions daily and 2 h water *ad libitum* after feeding. Sows that came into oestrus and ovulated between 3 and 7 days after weaning (n=115) were assigned to the study. The sows arrived from one commercial farm, from two synthetic lines (A, n=75 and B, n=40) which were terminal sire lines of fattening pigs (Dalland b.v.,

Merselo). The original breeds of the lines were: Pietrain, Large White and Dutch Landrace. All sows had a breeding value for litter size (range; -1.06 to 1.13 piglets) which was calculated based on their own and sib-relation performances. The sows were healthy upon clinical inspection. The number of sows from parity 1 to 7 was 36, 14, 36, 25, 3, 0 and 1, respectively.

Oestrus

Detection of oestrus was performed at intervals of 8 h, from 64 h after weaning until the end of oestrus. Every oestrus detection the back pressure test was performed, first in absence and then in presence of a boar. The time of onset of oestrus was defined as the first time a sow showed a standing response minus 4 h. The time of end of oestrus was defined as the last time a sow showed a standing response plus 4 h.

Ovulation

Ovulation was detected using transrectal ultrasonography as described by Soede *et al.* (1992). An annular array sector scanner (type 150V, Pie Medical b.v., Maastricht, The Netherlands) with a 5.0-7.5 MHz multiple scan angle transducer was used. A first check of the ovaries for presence and size of follicles (diameter of antrum > 4 mm) and corpora lutea was performed at approximately 70 h after weaning. From 16 h after the onset of oestrus, ovaries were checked at intervals of 4 h to estimate the moment of ovulation. Time of ovulation was defined as the first time when no follicles were counted minus 2 h. When the number of follicles was noticeably smaller than the previous scanning, ovulation was assumed to have just started, since ovulation takes on average 2 h in spontaneously ovulating sows (Soede *et al.*, 1992). Ovulation was confirmed by one additional scanning 4 h later.

Insemination

Artificial insemination was conducted once with doses of 1, 3 or 6 × 10^9 mixed sperm cells from three boars in 80 ml. The age of the inseminated sperm cells (time from collection) was on average 23 ± 7 h with a range of 11-36 h at the moment of insemination. Sperm quality was assessed for 3 consecutive days in samples of the sperm cell dosages (magnification × 200) by determining motile spermatozoa (0% to 100%) and quality of motility (0=very bad; 10=very good movement). At day 0, the

percentage of motile spermatozoa varied from 70% to 80%, and the quality of motility varied from 7 to 8. At day 2, the percentage of motile spermatozoa varied from 60% to 80% and the quality of motility varied from 6 to 7.

The moment of insemination depended on the predicted ovulation moment. The predicted ovulation moment was obtained from the interval weaning to the onset of oestrus, based on data from Soede *et al.* (1995a, b). The 1×10^9 dosage was inseminated at the predicted insemination to ovulation interval of 12-24 h. The 6×10^9 dosage was inseminated at the predicted insemination to ovulation interval of 24-36 h. The reference 3×10^9 dosage was inseminated at the predicted insemination to ovulation intervals of 12-24 h and 24-36 h. The sows were assigned randomly to the treatment groups.

Before insemination, sows were taken to the boar pen for 5 min boar stimulation. Artificial insemination took place for each sow in her own cage. During insemination, the backflow of semen was collected with a 200 ml cup which was weighed on a balance.

Embryonic development

Sows were slaughtered 120 ± 5 h (111-131 h) after ovulation and the embryos were collected immediately. Both oviducts were flushed with 15 ml Dulbecco's PBS (DPBS) from the infundibulum to the uterus. The oviducts were then separated from the uterus and each uterine horn was flushed twice with 30 ml DPBS to collect the embryos and oocytes. The quality and morphology of the recovered embryos and oocytes was assessed (magnification ×60) at the laboratory. Thereafter, embryos and oocytes were subjected to hypotonic treatment (0.6% w/v KCL, 0°C, 10 min) and subsequently placed on a fat-free glass slide. Small droplets of methanol/acetic acid (3/1 v/v) were added until disruption and spreading of the embryo (generally 1 cm^2) to allow the nuclei and spermatozoa to be counted (magnification × 200) after drying and staining with 10% (v/v) Giemsa (Merck, Damerstadt) in PBS. An oocyte was classified as unfertilised if the nuclei count was 0 or 1. Embryos with degenerated morphology and a small number of nuclei were classified as degenerated embryo. The remaining embryos were considered normal. Recovery rate per sow was determined as the percentage of embryos and oocytes recovered, based on the number of corpora lutea. The rate of normal and degenerated embryos and oocytes was determined on the basis of the total number of recovered embryos

and oocytes per sow. Fertilisation rate was determined as the percentage of normal and degenerated embryos among the total recovered embryos and oocytes per sow.

Statistical analyses

Data were analysed using SAS (1990). Data are presented as mean ± sd and a range (minimum-maximum) or least squares means ± sem when correction for specific factors was relevant.

The two synthetic lines used did not differ in any respect. The duration of oestrus (h) was analysed using the procedure GLM with the following factors: parity (2 classes) and weaning to oestrus interval (h). Parity was divided into 2 classes: 'young' sows (parity 1 and 2) and 'older' sows (parity 3 to 7). The time of ovulation (h) during oestrus was analysed using the procedure GLM with the factor duration of oestrus (h).

The influence of embryonic age (h) on embryonic development (cell cycles) was analysed using the procedure GLM. Embryonic development was expressed as the average number of cell cycles (^{2}log(nuclei count)) per sow. The embryonic age (h) was defined as the period between ovulation and slaughter of the sow.

Proportions of unfertilised, degenerated and normal embryos per sow underwent a normalising arcsine transformation before analysis (Snedecor and Cochran, 1989). These proportions of embryos were analysed using the Wilcoxon test of the NPAR1WAY procedure with the factors: insemination to ovulation interval (4 classes; 0-12 h, 12-24 h, 24-36 h and 36-48 h) and insemination dosages (3 classes: 1×10^9, 3×10^9 and 6×10^9 sperm cells).

The number of accessory sperm cells attached to normal embryos showed a lognormal distribution; therefore the ^{10}log(accessory sperm count per embryo) transformation was applied before the analysis. The accessory sperm cells were analysed using the Wilcoxon test of the NPAR1WAY procedure with the factors: insemination to ovulation interval (4 classes: 0-12 h, 12-24 h, 24-36 h and 36-48 h) and insemination dosages (3 classes: 1×10^9, 3×10^9 and 6×10^9 sperm cells). The influence of the continuous insemination to ovulation interval (h) on accessory sperm cells was analysed using the procedure GLM.

Sows were divided into 4 classes, depending on their fertilisation rate; 100-90, 90-80, 80-50 and 50-0%. The distribution of the proportions of fertilisation rates of the sows in the classes of insemination to ovulation interval (4 classes: 0-12 h, 12-24 h, 24-

36 h and 36-48 h) and insemination dosages (3 classes: 1×10^9, 3×10^9 and 6×10^9 sperm cells) was analysed with chi-square of the procedure FREQ.

The effect of sperm dosage (1, 3 and 6×10^9 sperm cells) on fertilisation rate was analysed in a continuous scale of insemination-ovulation intervals between 0 and 48 h. The analysis of variance on arcsine transformed fertilisation rates was done with the procedure GLM with the factors: insemination to ovulation time (0-48 h); sperm cell dosage (3 classses: 1×10^9, 3×10^9 and 6×10^9 sperm cells); parity (2 classes; 'young' and 'old' sows) and breeding value for litter size (-1.06 to1.13 piglets).

RESULTS

Animals

Of the 139 sows obtained, 19 sows did not show oestrus before 7 days after weaning, 3 sows had more than 20 ml backflow of semen during insemination, 1 sow had an insemination to ovulation interval of more than 50 h and 1 sow had one uterine horn. These sows were excluded from the study.

Oestrus and ovulation

In the 115 sows remaining, the weaning to oestrus interval was 92 ± 15 h, with a range of 65-132 h that was not affected by parity ($P>0.05$). The average duration of oestrus of all the sows was 59 ± 12 h, with a range of 24-88 h. The duration of oestrus was significantly shorter ($P=0.002$) in the young sows (parity 1 and 2: 55 ± 11 h, $n=50$) compared to the older sows (parity 3 to 7: 62 ± 12 h, $n=65$). The duration of oestrus was not significantly related to the weaning to oestrus interval ($P=0.18$).

Time of ovulation in hours after the onset of oestrus (OV) during oestrus was related to the duration of oestrus (h) (OEST): $OV = 8.6 + 0.5 \times OEST$ ($R^2=59\%$, $P<0.0001$, $n=114$). On average, ovulation took place at 68 ± 10% of the oestrus period.

Embryos and oocytes

Sows had a mean number of 21.1 ± 6.1 corpora lutea. The number of recovered embryos and oocytes compared to the number of corpora lutea varied from 55% to 100%, with a mean of 89 ± 6.8%.

Fertilisation rate varied between 0% and 100% per sow. Degenerated embryos appeared in 36% of the sows; the mean percentage of degenerated embryos in all sows was 4 ± 8%. The number of degenerated embryos was not affected by insemination dosage (P=0.29) nor by the insemination to ovulation interval (P=0.19). The proportion of unfertilised embryos was similar to the reciprocal of the proportion of normal embryos per sow, because of the small number and equal distribution of degenerated embryos.

The mean age of the embryos was 120 ± 4.5 h and varied between 111 and 131 h. The average development of the normal embryos per sow varied between 3.7 and 7.3 cell cycles. The development of normal embryos was not significantly affected by the insemination dosage (P=0.89) or insemination to ovulation interval classes (P=0.45). The variation in embryo development between sows was related to the age of the embryos (AGE, h); cell cycles = -1.17 + 0.055 × AGE (R^2=14%, P<0.0001, n=107); at an embryo age of 120 h, the mean development was 5.4 cell cycles.

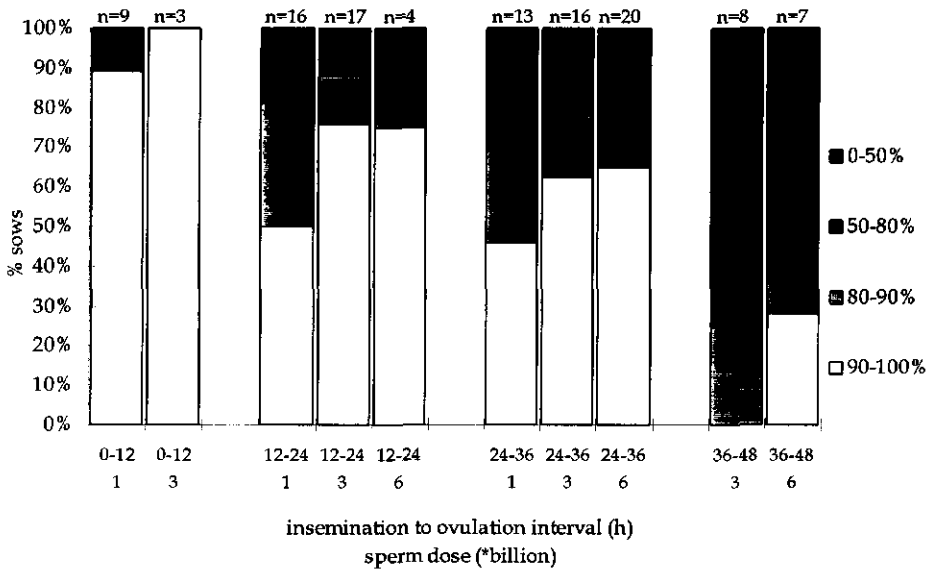

Figure 1. The percentage of sows displaying bad (0-50%), poor (50-80%), moderate (80-90%) and good (90-100%) fertilisation rates after artificial insemination with 1, 3 or 6 × 10^9 sperm cells with insemination to ovulation interval classes of 10-12 h, 12-24 h, 24-36 h and 36-48 h.

Fertilisation results in 12 h insemination to ovulation interval classes

The percentage of normal embryos varied considerably in all the insemination to ovulation interval classes from 0 to 100%. A shift of good fertilisation to poor and bad fertilisation was observed when the insemination to ovulation interval increased (Figure 1). The 4 classes of fertilisation in Figure 1 are: good fertilisation (100-90% normal embryos), moderate fertilisation (90-80% normal embryos), poor fertilisation (80-50% normal embryos) and bad fertilisation (50-0% normal embryos). A shift of good fertilisation to poor and bad fertilisation was seen when the insemination to ovulation interval increased.

The median percentage of normal embryos was not significantly different between insemination dosages 1×10^9 and 3×10^9 in the insemination to ovulation interval of 12-24 h (Table 1). However, the percentage of sows in the 4 fertilisation classes in the insemination to ovulation interval of 12-24 h were distributed significantly different ($P<0.05$) between the dosage 1×10^9 and 3×10^9 sperm cells (Figure 1). The dosage of 1×10^9 sperm cells resulted in 26% fewer sows with good fertilisation results (100-90% normal embryos) and 19% more sows with moderate fertilisation results (80-90% normal embryos) compared to the dosage 3×10^9 sperm cells (Figure 1).

Table 1. Fertilisation results in sows that were inseminated at 12-24 h and 24-36 h before ovulation with an insemination dosage of 1×10^9, 3×10^9 and 6×10^9 sperm cells.

	12-24 h		24-36 h	
Dosage	1×10^9	3×10^9	3×10^9	6×10^9
Sows (n)	16	17	16	20
IO interval	19 ± 3	20 ± 3	29 ± 4	30 ± 3
Normal embryos (%)				
Mean	89 ± 15	88 ± 28	83 ± 22	83 ± 26
Median	95	100	95	97
Range	55-100	0-100	42-100	6-100
Accessory sperm count				
Mean	30 ± 41	22 ± 20	16 ± 18	11 ± 9
Median	11	17	8	11
Range	1-147	2-66	0-57	0-28

Within an insemination to ovulation interval, there were no significant differences between dosages ($P>0.05$).

The median percentage of normal embryos was not significantly differently between insemination dosages 3×10^9 and 6×10^9 in the insemination to ovulation interval of 24-36 h (Table 1). The percentage of sows in the 4 fertilisation classes in the insemination to ovulation interval of 24-36 h were not distributed significantly different ($P<0.05$) between the dosage 3×10^9 and 6×10^9 sperm cells (Figure 1).
However, the fertilisation results of sows inseminated with 1×10^9 sperm cells in this insemination to ovulation interval were distributed significantly different compared with the higher sperm dosages ($P<0.05$). These group of sows inseminated with 1×10^9 sperm cells had a higher percentage sows (23%) with moderate fertilisation results (Figure 1).

Insemination between 36-48 h before ovulation with 3×10^9 and 6×10^9 sperm cells did not result in a significantly different distribution of sows among the 4 fertilisation classes (Figure 1). However, the dosage 6×10^9 sperm cells still resulted in 28% sows with good fertilisation results (100-90% normal embryos), whereas the dosage 3×10^9 resulted in 0% sows with good fertilisation.

Percentage normal embryos in a continuous insemination to ovulation interval (0-48 h)

An analysis of variance on the transformed percentage of normal embryos per sow showed significant relation ($R^2=0.28$; $P<0.0001$) with the following variables. The effect of insemination to ovulation interval is negative and highly significant ($b= -0.019 \pm 0.003$; $P<0.0001$), which means that an increase of the insemination to ovulation interval by 24 h resulted in a decrease of 20% in the percentage of normal embryos. Sperm dosage tends to affect the percentage of normal embryos ($P=0.096$); the dosages 1, 3 and 6×10^9 sperm cells resulted in respectively $78 \pm 0.3\%$, $84 \pm 0.3\%$ and $91 \pm 0.4\%$ normal embryos (LSM ± SEM). The 'young' sows (parity 1 and 2) had significantly lower ($P=0.026$) percentages normal embryos (79.1%) than the 'old' sows (parity 3 to 7) (88.8%). The genetic background of the sows, based on the breeding value for litter size, also influenced the percentage of normal embryos in the sows ($b=0.21 \pm 0.05$; $P=0.0026$). An increase in breeding value of 1 piglet increased the percentage of normal embryos by 4.5%.

Accessory sperm count

The accessory sperm count on normal embryos is highly variable among and within sows and within the dosages 1, 3 and 6×10^9 sperm cells (Figure 2). In the

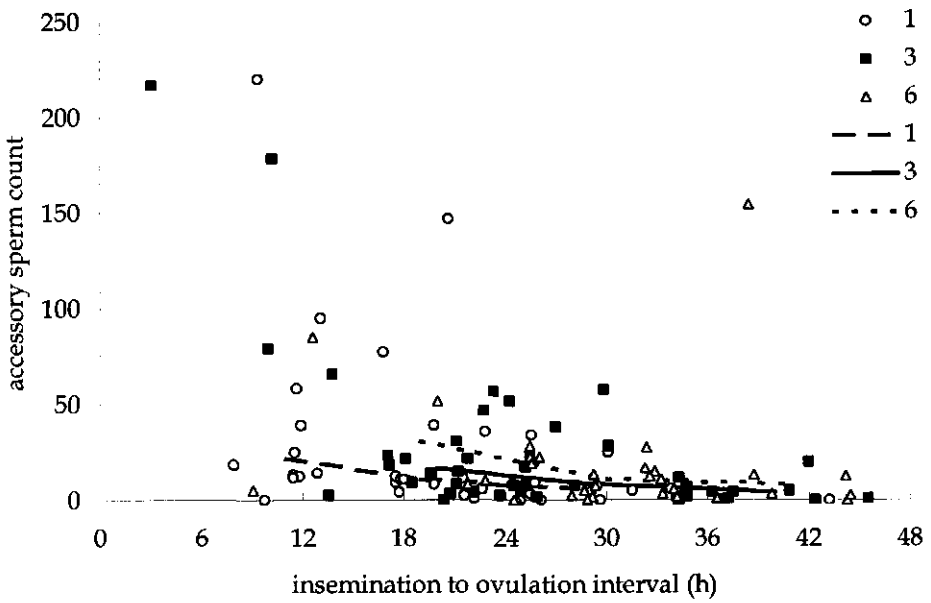

Figure 2. The accessory sperm count on normal embryos per sow for the dosages 1, 3 and 6 × 10^9 sperm cells (1, 3 and 6, respectively). The lines join the median accessory sperm count in the insemination to ovulation interval classes of 0-12 h, 12-24 h, 24-36 h and 36-48 h for the three sperm dosages.

insemination to ovulation interval of 0-12 h the dosages 1, 3 or 6 × 10^9 sperm cells had a median accessory sperm count: 22, 178 and -, respectively; in the interval of 12-24 h: 11, 17 and 31, respectively; of 24-36 h: 6, 8 and 11, respectively and of 36-48 h: -, 4 and 8. The accessory sperm count was not significantly different among the insemination dosages in any of the insemination to ovulation interval classes. Sows inseminated with 1 × 10^9 sperm cells had a significantly higher ($P<0.05$) median accessory sperm count in the insemination to ovulation intervals of 0-12 h and 12-24 h (22 and 11, respectively) compared to the insemination to ovulation interval 24-36 h (6). Sows inseminated with 3 × 10^9 sperm cells had a significantly higher ($P<0.05$) median accessory sperm count in the insemination to ovulation interval of 0-12 h (178) compared to the insemination to ovulation intervals of 12-24 h, 24-36 h and 36-48 h (17, 6 and 4, respectively). Sows inseminated with 6 × 10^9 sperm cells had a significantly higher ($P<0.05$) median accessory sperm count in the insemination to

ovulation intervals of 12-24 h and 24-36 h (31 and 11) compared to the insemination to ovulation interval 36-48 h (8). The median number of accessory sperm cells in sows with 100% normal embryos (n=50) was 18, with a range of 1.6-220 accessory sperm cells.

The accessory sperm count decreased significantly with an increase in the insemination to ovulation interval. The effect of insemination to ovulation interval in hours (IO) on accessory sperm count was (ASPERM): $^{10}\log(\text{ASPERM}) = 1.63 - 0.028 \times \text{IO}$; ($R^2$=0.24, P<0.0001, n=101).

DISCUSSION

Fertilisation rates at day 5 of gestation were not significantly affected by insemination dosage 1×10^9 versus 3×10^9 in the insemination to ovulation interval of 12-24 h and not affected by insemination dosage 3×10^9 versus 6×10^9 in the insemination to ovulation interval of 24-36 h. However, small consistent differences were seen, both the median percentage of normal embryos and the median accessory sperm count increased with an increase in insemination dosage in all the insemination to ovulation classes 0-12 h, 12-24 h, 24-36 h and 36-48 h.

A positive effect of increased number of sperm cells at insemination on fertilisation was expected. For example, Baker *et al.* (1968) compared insemination dosages 1×10^9 and 5×10^9 sperm cells in 10 gilts inseminated 6 to 8 h before ovulation and found 24% versus 73% fertilised eggs at 6 to 8 h after ovulation, respectively. The *in vivo* results of the expected increased number of sperm cells are supported by experiments *in vitro*. Lefebvre and Suarez (1996) showed that the number of sperm cells bound per mm^2 isthmic epithelium in vitro increased by using more sperm cells in the medium. Increased sperm numbers at insemination *in vivo* may result in an increased length and thereby area of the sperm reservoir, and also a higher density of sperm cells per mm^2. Therefore, it seems logical that the functional filling of the sperm reservoir in pigs can be increased by increasing the number of inseminated sperm cells. In the present study, no evidence was found for an increased fertilisation rate or accessory sperm count due to an increased number of sperm cells at insemination.

If a greater number of spermatozoa is deposited in the reservoir with a higher insemination sperm dosage, the lack of dosage effects might be explained by the release pattern from the reservoir. After filling the reservoir, the release of sperm cells from the reservoir is exponential in the first hours after insemination. Therefore, the difference between sperm dosages may be visible only when the insemination to ovulation interval is short (<12 h). Baker *et al.* (1968) inseminated gilts 6-8 h before ovulation with 1×10^9 to 5×10^9 sperm cells and found a significant increase in the median accessory sperm count from 3 to 130. In the present study, differences in accessory sperm count between dosages became progressively smaller with an increasing insemination to ovulation interval. The lack of effect of sperm dosages on fertilisation rates does not mean that sperm reservoirs are not filled with different numbers of sperm cells due to different sperm dosages. The regulation of the number of sperm cells bound to isthmic epithelium is still not understood. More research on reservoir filling, capacity and release of the sperm reservoir is necessary to understand these mechanisms better.

It has been suggested that large numbers of accessory sperm cells positively affect embryo quality (Hunter and Wilmut, 1984; DeJarnette *et al.*, 1992; Nadir *et al.*, 1993; Saacke *et al.*, 1994 (review)). In the present study, the variation in accessory sperm count between animals is very high. In sows inseminated 0-12 h before ovulation, the mean accessory sperm count ranged from 1 up to 216. Many sows with 100% normal developed embryos had a low accessory sperm count. This finding suggests that there is not a direct relationship between embryo quality and accessory sperm count. The accessory sperm count is strongly related to the insemination to ovulation interval. In the present study, large numbers of accessory sperm cells (>75) attached to normal developed embryos were found only when the insemination to ovulation interval was short (<18 h). A large number of accessory sperm cells is an indication of a short insemination to ovulation interval. A similar relation between accessory sperm count and insemination to ovulation interval was found in the studies of Soede *et al.* (1995a, b). Therefore, the cause of a higher embryo quality (good developed embryos) mentioned by Hunter and Wilmut (1984), DeJarnette *et al.* (1992), Nadir *et al.* (1993) and Saacke *et al.* (1994) could be the result of a short insemination to ovulation interval and not of the number of accessory sperm cells beforehand. For a correct interpretation of the relation between accessory sperm count and embryo quality it is necessary to know the insemination to ovulation interval.

In the present study, a part of the variation in fertilisation rate had a genetic basis. The percentage of normal embryos was positively related ($P<0.01$) to the breeding value for litter size of the sows. This means that the fertilisation rate at day 5 of gestation is related to the number of piglets the sow potentially can produce. An enlargement of the optimal insemination to ovulation interval is seen in sows with a high breeding value. At present, there are no studies available describing relations between genetic potential for litter size in sows and their fertilisation rate on day 5 of gestation.

In conclusion, no significant effect of the sperm dosage of 1, 3 and 6×10^9 sperm cells was seen on fertilisation rate, nor on the accessory sperm count. The insemination to ovulation interval has a significant effect on the fertilisation rate and accessory sperm count. The process and regulation of filling, capacity and release of sperm cells in the reservoir are still not understood and require further study.

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2.2 Backflow of semen

Semen backflow after insemination and its effects on fertilisation in sows

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Semen backflow after insemination and its effects on fertilisation in sows

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ABSTRACT

The aim of the present study was to investigate the volume of and number of spermatozoa in semen backflow during and after insemination, and the effect of backflow on fertilisation results assessed at day 5 of pregnancy. Multiparous sows (n=140) were artificially inseminated with either 1, 3 or 6×10^9 mixed spermatozoa from three boars in a constant volume of 80 ml. Backflow of semen was measured three times: during insemination (M1); during the first half hour after insemination (M2); and from 0.5 h until about 2.5 h after insemination (M3). Transrectal ultrasonography was performed at intervals of 4 h to determine the time of ovulation. Sows were sacrificed at 120±0.4 h after ovulation to assess the results of fertilisation. Every sow had some backflow and the variation in volume, and number of spermatozoa within the backflow was high. The average semen backflow within 2.5 h after insemination was 70±3.4% of the volume and 25±1.4% of the spermatozoa of the inseminated dosage. The concentration of the backflow (% of the inseminated dosage) decreased with time after insemination from 65% at M1 to 40% and 26% at M2 and M3, respectively. The correlations between volume and number of spermatozoa were high: r=0.97, r=0.73 and r=0.81 in M1, M2 and M3, respectively. More than 5% of the inseminated spermatozoa in backflow during insemination affected fertilisation negatively in those sows inseminated with 1×10^9 spermatozoa ($P<0.05$). Backflow after insemination had no effect on fertilisation results ($P>0.05$). Timing of insemination relative to ovulation and oestrus were not related to backflow during or after insemination ($P>0.05$). Of the sows which had backflow, those of parity 1 tended to have the highest proportion of sows with more than 5 ml backflow (47%; n= 8 of 17) compared with sows from parity 2 and higher (24%; n=14 of 59) ($P=0.075$).

It was concluded that excessive backflow of semen during insemination had a negative effect on fertilisation results when sows where inseminated with only 1×10^9 spermatozoa. Causes of variation in backflow between sows were not clearly identifiable.

Key words: Pig, Reproductive technology, Artificial insemination, Fertilisation, Semen backflow, Retrograde spermatozoa

INTRODUCTION

Sufficient fertile spermatozoa should be present in the isthmus of the oviduct at ovulation for optimal fertilisation. A large volume of semen (natural mating: up to 300 ml, and artificial insemination: 80-100 ml) containing a large number of spermatozoa (natural mating: up to 60×10^9 and artificial insemination: up to 3×10^9) is deposited directly into the uterus (Garner and Hafez, 1993) at insemination. A limited volume of semen is necessary for good fertilisation results. Baker et al. (1968) compared the results of inseminations with 20, 100 and 200 ml semen and concluded that gilts inseminated with 100 ml semen had a significantly higher proportion of oocytes fertilised than gilts inseminated with 20 or 200 ml semen.

Spermatozoa are transported for 1 to 2 m (length of uterine horn) through the female genital tract in the fluid of the inseminated dosage. Longitudinal contractions of the uterus are mainly responsible for transport of the spermatozoa (Zerobin, 1968; Bower, 1974). Spermatozoa are already found in the oviducts 5 min after artificial insemination (Viring et al., 1980; Baker and Degen, 1972), where a sperm reservoir is formed in the caudal region of the isthmus (Hunter, 1981; Overstreet et al., 1980; Suarez et al., 1991).

The fluid and number of spermatozoa of the inseminated dosage decreases rapidly in the uterus, during the first hours after insemination (First et al., 1968; Viring and Einarsson, 1981)). Loss of semen soon after insemination (semen backflow) could hinder optimal transport of spermatozoa to the oviduct and decrease the number of spermatozoa available for fertilisation.

The aim of the present study was to investigate the volume of and number of spermatozoa in semen backflow during and after insemination, and the effect of backflow on fertilisation results assessed at 5 days of pregnancy.

MATERIALS AND METHODS

Data were used from an experiment which was designed to describe the effects of sperm dosage and the time of insemination relative to ovulation on fertilisation rate and accessory sperm count in sows (Steverink et al., 1997). The first batch was excluded since semen backflow was not measured and data from sows of a third genetic line (C) were added. Data on oestrus duration, weaning to oestrus interval and time of ovulation during oestrus have been described by Steverink et al. (1997).

Animals and housing

Every second week (batch) for a period of 14 weeks, 9 to 22 sows (161 in total) were obtained on the day their litter was weaned. The sows were housed individually in stalls at the experimental farm, and received a total of 2.5 kg of a commercial sow diet (12.9 MJ ME kg^{-1}) in two portions daily, and 2 h water ad libitum after feeding. Sows that came into oestrus and ovulated between 3 and 7 days after weaning (n=140) were then assigned to the study. The number of sows from parity 1 until 8 was 36, 25, 39, 23, 7, 3, 5 and 2, respectively. There were 19 sows that did not show oestrus within 7 days of weaning, one sow had an insemination to ovulation interval of more than 50 h, and one sow had only one uterine horn to provide 140 sows suitable for investigation. The sows were derived from two commercial farms and from three synthetic lines (A: n=67, B: n=34 and C: n=39); A and B were terminal sire lines of fattening pigs, and C was a terminal dam line of fattening pigs (Dalland b.v., Merselo).

Oestrus

Detection of oestrus was performed each day at intervals of 8 h (8:00, 16:00 and 24:00 h), from 64 h after weaning until the end of oestrus. The back pressure test was performed every oestrus detection, firstly in the absence and then in the presence of the boar. The onset of oestrus was defined as the first time a sow showed a standing response minus 4 h. The end of oestrus was defined as the last time a sow showed a standing response plus 4 h.

Ovulation

Detection of ovulation was done by transrectal ultrasonography as described by Soede et al. (1992). An annular array sector scanner (type 200V, Pie Medical b.v., Maastricht, The Netherlands) with a 5-7.5 MHz multiple scan angle transducer was used. A first check of the ovaries for the presence and size of follicles (diameter of antrum > 4 mm) and corpora lutea was performed approximately 70 h after weaning. From 16 h after the onset of oestrus, ovaries were checked at intervals of 4 h to estimate the time of ovulation. Time of ovulation was defined as the first time when no follicles were observed minus 2 h. When the number of follicles was noticeably lower than for the previous scan, ovulation was assumed to have started, as ovulation spans an average period of 2 h in spontaneously ovulating sows (Soede et al., 1992). Ovulation was confirmed by an additional scan 4 h later.

Insemination

Artificial insemination was conducted once with either 1, 3 or 6×10^9 mixed spermatozoa from three boars in a constant volume of 80 ml. The age of the inseminated spermatozoa (time from collection) was less than 36 h at the time of insemination. Sperm quality was assessed microscopically for 3 consecutive days after collection, in samples taken from the inseminate (magnification ×200); motile spermatozoa (0% to 100%) and quality of motility (0=very bad; 10=very good linear straight forward movement). The percentage of motile spermatozoa varied from 70% to 80%, and the quality of motility varied from 7 to 8 on the first day after semen collection. On day 2, the percentage of motile spermatozoa varied from 60% to 80% and the quality of motility varied from 6 to 7.

Prior to insemination, sows were taken in front of the boar pen for 5 min boar stimulation. Subsequently, each sow was artificially inseminated in her own cage making use of a 10 kg bag on the sows back. Sows were inseminated at different times after the onset of oestrus as described by Steverink et al. (1997). This resulted in insemination-to-ovulation intervals (IO) ranging from 0-48 h. For analyses, the IO was divided in an optimal and suboptimal class: 0-24 h and 24-48 h, respectively (Soede et al., 1995).

Backflow of semen

Semen backflow was collected at three time points: during insemination (M1); during the first half hour after insemination (M2); and backflow from 0.5 h onwards after insemination (M3). The average duration of M3 was 1.9 ± 0.8 h (range: 0.3 - 4.3 h). Backflow during insemination was collected with a 200 ml cup. The backflow after insemination was collected into a human colostomy bag (ConvaTec, Woerden, The Netherlands). The colostomy bag was fixed around the vulva of the sow and secured with tape. When a sow had urinated into the colostomy bag or the colostomy bag was damaged, the value was deleted from the data. The cup (M1) or the colostomy bag (M2 and M3) were emptied into a tube and weighed on a balance (± 1 g). Then the semen backflow was frozen until further processing. The backflow samples were mixed on a vortex after thawing and diluted to 1:20 (1 billion dosage) and 1:50 (3 and 6 billion dosages). The sperm concentrations were assessed in duplicate per sample using a Bürker counting chamber.

Embryonic development

Sows were slaughtered 120 ± 0.4 h (111-131 h) after ovulation and the embryos collected immediately. Both oviducts were flushed with 15 ml Dulbecco's PBS (DPBS) from the infundibulum to the uterus. Subsequently, the oviducts were separated from the uterus and each uterine horn flushed twice with 30 ml DPBS to collect the embryos and oocytes. The quality and morphology of the recovered embryos and oocytes were assessed (magnification ×60) microscopically at the laboratory. Thereafter, embryos and oocytes were subjected to hypotonic treatment (0.6% w/v KCL, 0°C, 10 min) and subsequently placed on a fat-free glass slide. Small droplets of methanol/acetic acid (3/1 v/v) were added until disruption and spreading of the embryo occurred (generally 1 cm^2). The nuclei and spermatozoa were counted (magnification ×200) after drying and staining with 10% Giemsa in PBS. An oocyte was classified as unfertilised if the nuclei count was zero or one. Embryos with degenerated morphology together with a low number of nuclei, were classified as degenerated embryo. The remaining embryos were considered normal. Recovery rate per sow was determined as the percentage of embryos and oocytes recovered, based on the number of corpora lutea. The rate of normal and degenerated embryos and oocytes was determined on the basis of the total number of recovered embryos and oocytes per sow. Fertilisation rate was determined as the percentage of normal

and degenerated embryos in the total number of recovered embryos and oocytes per sow.

Statistical analyses

Data were analysed using SAS (1996). Data are presented as mean±se and a range (minimum-maximum).

Backflow volume, backflow sperm cell concentration and number of spermatozoa are expressed as % of the inseminated dosage to make the different insemination dosages comparable. The effects of sperm dosage on backflow volume, backflow sperm cell concentration and relative number of spermatozoa were analysed using the GLM procedure, as was the effect of the duration of M3 (total measure time) on the volume and number of spermatozoa in the backflow.

In the 6 combinations of sperm dosage (3 classes: 1, 3 or 6×10^9 spermatozoa) and insemination to ovulation intervals (IO) (2 classes: 0-24 h and 24-48 h), the effect of semen backflow on percentage normal embryos was analysed with Fisher's exact test (2x2 factorial design; Kendall and Stuart, 1979) using the FREQ procedure. Sows with backflow were divided in two classes: 'high' representing the 20% of sows with the highest percentage of spermatozoa in backflow; and 'low' representing the 80% sows with the lowest percentage of spermatozoa in backflow. This resulted in the following percentage of backflow in the two classes: for backflow during insemination; M1: 0 to 4.8% and more than 4.8% spermatozoa; for backflow after insemination: M2: 0 to 23% and more than 23% spermatozoa; and for M3: 0 to 14% and more than 14% spermatozoa. For percentage normal embryos, the border of the classes was taken according to Steverink et al. (1997): 'low' was 0-80% and 'high' was 80-100% normal embryos.

The difference in distribution of sows over the two parity classes (parity 1 vs. parity 2 to 8) over the two classes of backflow was analysed with the Fisher's exact test of the FREQ procedure.

Table 1. The volume and number of spermatozoa in the semen backflow (mean±se and range) during insemination (M1) and after insemination (M2, M3) with 80 ml and 1, 3 or 6×10^9 spermatozoa[1] for sows having backflow, expressed as a percentage of the inseminated dosage.

		Volume (%)		Spermatozoa (%)	
Backflow[2]	n/n_t[3]	Mean ± se	Range	Mean ± se	Range
M1	76/120	7 ± 1.1	1 - 56	8 ± 1.3	0.3 - 50
M2	110/112	31 ± 1.7	3 - 76	14 ± 1.0	0.3 - 79
M3	78/80	36 ± 2.6	1 - 94	9 ± 0.8	0.3 - 30

[1] *The average of the dosages 1, 3 or 6×10^9 spermatozoa are presented since no significantly differences were found between dosages.*

[2] *Measurement: M1: backflow during insemination; M2: backflow 0-0.5 h after insemination; M3: backflow 0.5-2.5 h after insemination.*

[3] *n/n_t: sows with backflow (>0%) / all sows.*

RESULTS

General

Of the 140 sows used, 120 sows were successfully measured for M1, 112 sows for M2 and 80 sows for M3. There were 59 sows with a complete record of 3 volume measurements of backflow (M1, M2 and M3). From these 59 sows, 54 sows had a complete record of the number of spermatozoa in the backflow.

Volume of the semen backflow

Backflow was seen in each of the 140 sows: 63% of the sows had backflow during insemination (M1); 98% had backflow from 0-0.5 h after insemination (M2); and 98% had backflow from 0.5-2.5 h after insemination (M3) (Table 1). In the sows with backflow, the volume of backflow was very variable in all three measurements; M1 ranged from 1 to 56%; M2 from 3 to 76%; and M3 from 1 to 94% (Table 1). The volume of backflow during M3 was not related to the duration of collection ($P>0.05$). The overall volume of backflow of sows with backflow (> 0 ml), was on average 7±1.1%, 31±1.7% and 36±2.6% of the 80 ml during (M1) and after insemination (M2, M3), respectively. The inseminated sperm dosage (1, 3 or 6×10^9 spermatozoa) had no effect on the volume of backflow in either of the three measurements ($P>0.05$).

The distribution of sows (with a complete record) in backflow volume classes of 10% are shown in Figure 1. The total volume of backflow after insemination up to

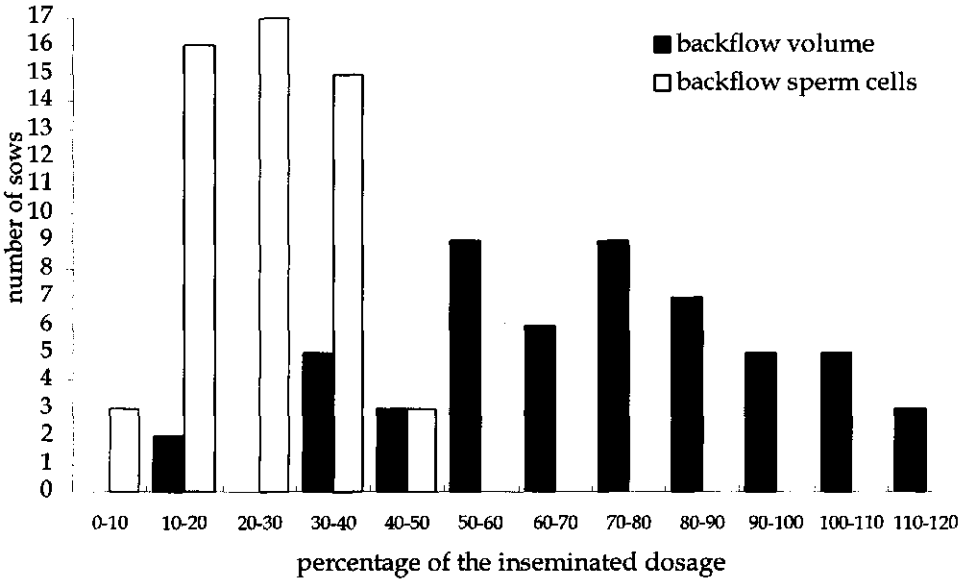

Figure 1. Number of sows in classes of volume (n=59) and spermatozoa (n=54) in the backflow during and after insemination relative to the inseminated dosage, where all sows had a complete record (M1+M2+M3).
Where, M1: backflow during insemination; M2: backflow 0-0.5 h after insemination; M3: backflow 0.5-2.5 h after insemination.

2.4±0.1 h was, 70±3.4% of the inseminated dosage recovered within a range of 17 to 120%.

The number of sows with backflow during insemination was not influenced by parity (P>0.05). Of the sows which had backflow, a larger proportion of those of parity 1 tended to have more than 5 ml backflow (47%; 8 of 17) compared to those of parity 2 and higher (24%; 14 of 59) (n=76; P=0.075). Of the 5 sows which had more than 20 ml backflow during insemination (M1), 4 sows were from parity 1 and 1 sow was from parity 2 or higher which was significant different (n=76; P<0.01). The interval from onset of oestrus to insemination or the interval from insemination to ovulation were not related to the volume of backflow in any of the three measurements (P>0.05).

Number of spermatozoa in the semen backflow

The number and concentration of spermatozoa in the backflow was related to the sperm dosage, but the relative number of spermatozoa and the relative sperm cell

concentration (% of the inseminated dosage), were not affected by sperm dosage (P>0.05). The average concentration of the spermatozoa in the backflow compared to the inseminated dosage was 65±2.6%, 40±1.5% and 26±1.4% at M1, M2 and M3, respectively. The correlation between volume and number of spermatozoa was high: r=0.97, r=0.73 and r=0.81 at M1, M2 and M3, respectively. The average percentage of spermatozoa in the backflow was 8±1.3%, 14±1.0% and 9±0.8% at M1, M2 and M3, respectively (Table 1). The highest number of spermatozoa was retrieved in period M2 (14±1.0%). The average duration of measurement of M3 was 1.9±0.1 h, but the relative number of spermatozoa was not related to the duration of M3 (P>0.05).

The distribution of sows (with a complete record) of backflow classes of 10% are shown in Figure 1. The average total number of spermatozoa in the backflow up to 2.4±0.1 h after insemination, was 25±1.3% of the inseminated dosage, with a range of 3-48%.

The effect of backflow on fertilisation results

Sows had a mean number of 20.8±0.5 corpora lutea. The number of recovered embryos and oocytes, compared to the number of corpora lutea varied from 55% to 108%, with a mean of 88.4±0.7%.

Fertilisation rate varied from 0% to 100% per sow. Degenerated embryos were seen in 36% of the sows; the mean percentage of degenerated embryos in all sows was 4±0.7%. The proportion of fertilised ova was similar to the proportion of normal embryos per sow, because of the low number and equal distribution of degenerated embryos.

A negative effect was found with a high amount of backflow on the percentage normal embryos (P<0.05) when 1×10^9 spermatozoa was inseminated (Table 2). In the 0-24 h IO, 96% of the sows with low backflow had a high percentage of normal embryos (median=100%), while only 50% of the sows with high backflow had a high percentage of normal embryos (median=68%) (P=0.035; Table 2). In the 24-48 h IO, 82% of the sows with low backflow had a high percentage normal embryos (median=93%), while the sows with high backflow all had a low percentage of normal embryos (median=46%) (P<0.01: Table 2). This negative effect of backflow during insemination (M1) was not seen in sows inseminated with 3 or 6×10^9 spermatozoa (P>0.05). The amount of backflow after insemination (M2 and M3) had no effect on the percentage normal embryos in any of the 3 inseminated dosages (P>0.05; Table 2).

Table 2. Influence of backflow (low vs. high[1]) during (M1) and after insemination (M2, M3) on median percentage normal embryos (Norm) for dosages 1, 3 and 6×10^9 spermatozoa in the 0-24 h and 24-48 h insemination to ovulation intervals (IO) for sows.

Sperm dosage	Backflow[2]	0-24h IO interval				24-48h IO interval			
		Low		High		Low		High	
		n	Norm (%)	n	Norm (%)	n	Norm (%)	n	Norm (%)
1×10^9	M1	28	100[a]	4	68[b]	17	93[a]	5	46[b]
	M2	22	100	5	100	18	85	4	90
	M3	14	100	2	100	9	70	4	98
3×10^9	M1	12	98	3	96	17	68	4	89
	M2	6	98	4	97	15	71	5	100
	M3	7	95	1	96	13	88	3	53
6×10^9	M1	2	92	1	-	21	90	6	97
	M2	4	100	0	-	21	100	3	42
	M3	1	-	0	-	17	72	5	100

[1] *Backflow: high: 20% of the sows with the highest relative number of spermatozoa in backflow. low: 80% of the sows with the lowest relative number of spermatozoa in backflow.*

[2] *Measurement: M1: backflow during insemination; M2: backflow 0-0.5 h after insemination; M3: backflow 0.5-2.5 h after insemination.*

[a,b] *Within IO interval, different superscripts indicate significant differences between sows with low and high backflow according to the Fisher exact test where percentage normal embryos was divided in 2 classes: 0-80% and 80-100% ($P<0.05$).*

DISCUSSION

Backflow of semen may be a normal physiological process in pigs, since semen backflow was seen with every sow. The volume of backflow (70% of the inseminated volume) was relatively high compared to the number of spermatozoa in backflow (25% of the inseminated spermatozoa) during the 2.5 h after insemination. In this study, the percentage of spermatozoa in backflow was similar to the spermatozoa in backflow recovered after natural mating in a study of Viring and Einarsson (1981). They concluded that approximately one-third of the spermatozoa in the ejaculate

disappeared by backflow within 2 h after mating. Although the variation in total amount of backflow was variable between sows, the correlation between the number of spermatozoa and the volume of backflow was high within the three measurements (M1, M2 and M3). Therefore the volume of backflow was an indication of the number of spermatozoa in backflow. The total proportional volume of backflow within 2.5 h after insemination varied between 20% to 120%. Some sows (n=5) had more than 100% backflow, probably as a result of secretion of mucus. It was not due to urine as evidenced by the small amount, colour and smell of the backflow.

The amount of backflow during and after insemination was not related to the timing of insemination during oestrus. Backflow of semen is therefore not an indicator of incorrect timing of insemination relative to the time of ovulation. Baker and Degen (1972) found a correlation between size of uterus and number of spermatozoa in backflow. This may explain why relatively more parity 1 sows had a high volume of backflow (>20 ml) during insemination than older parity sows. Also, variation in contraction activity of the uterus may cause variation in backflow between sows. The uterus shows myometrial activity during oestrus (Claus et al., 1989) and increased uterine contractions, both in frequency and amplitude after natural mating and artificial insemination (Zerobin, 1968; Bower, 1974). Variation between pigs was found in these studies.

Spermatozoa seem to stay progressively in the reproduction tract of pigs, since the concentration of spermatozoa in backflow decreased when time from insemination to backflow collection increased from 0 to 2.5 h. The concentration of spermatozoa in backflow during insemination was high (M1=65% of the concentration in the inseminated dosage) compared to the backflow after insemination (M3= 26% of the concentration in the inseminated dosage). Baker and Degen (1972) found a similar decrease of sperm cell concentration in uterine horns and oviducts after flushing. The concentration decreased from 62×10^6 spermatozoa/ml to 11×10^6 spermatozoa/ml from 15 to 60 minutes after insemination was performed with 19 to 24×10^9 spermatozoa in 100 ml fluid. Histological examination of uterine epithelium after mating demonstrated that spermatozoa do adhere to uterine cilia, glandular tubules and surface epithelium (Lovell and Getty, 1968). Phagocytosis seems to be responsible for the removal of the largest part of the inseminated spermatozoa, as Pursel et al. (1978) reported that gilts slaughtered 2 h after insemination had high numbers of leukocytes in the uterus. Summarising, from these studies it would seem that a large part of the inseminated

volume disappeared from the uterus due to backflow and that the inseminated spermatozoa disappeared from the uterus due to both backflow and phagocytosis.

Excessive backflow during insemination affected fertilisation negatively, though only for the sows inseminated with 1×10^9 spermatozoa. Although the number of sows was not large, it could be concluded that suboptimal circumstances like a combination of low dosage and loss of spermatozoa due to backflow during insemination, may lead to sub-optimal fertilisation results. The fluid of the inseminated dosage could be a limiting factor for optimal fertilisation results. The fluid of an insemination dosage is the medium for transporting the spermatozoa. Baker et al. (1968) compared the results of insemination with 5×10^9 spermatozoa in 20, 100 and 200 ml semen and concluded that gilts inseminated with 100 ml semen had a significantly higher proportion of eggs fertilised and more sperm attached to the zona pellucida than gilts inseminated with 20 and 200 ml semen. Stratman and Self (1960) found a requirement of at least 50 ml to obtain suitable results with AI. Loss of semen during insemination could hinder the optimal transport of spermatozoa to the oviduct due to the reduced volume in the uterus.

No effect of backflow after insemination (M2 and M3) was found on fertilisation results. The variation in the volume of backflow in M2 was high and ranged from 0-76% of the inseminated dosage. Spermatozoa have been found in the oviduct within 5 min after insemination (Baker and Degen, 1972; Viring et al., 1980). Rapid sperm transport due to uterine contraction will commence immediately after insemination. From our study it may be concluded that in some cases, enough spermatozoa are in the top of the uterine horns within 30 min after insemination, since in some sows more than 80% of the inseminated fluid disappeared through the vulva within 30 min and good fertilisation results were still obtained. Backflow after insemination, does not seem to affect the number of spermatozoa in the sperm reservoir. Even preventing backflow from the uterus with a cotton tampon or a plastic plug placed into the cervix after insemination did not increase the number of spermatozoa recovered from the uterine horns and utero-tubal-junction 4 h after insemination (Pursel et al., 1982).

In conclusion, backflow during and after insemination was highly variable between sows. Excessive backflow during insemination (more than 5% of the inseminated dosage) had a negative effect on fertilisation results when sows were inseminated with 1×10^9 spermatozoa, but this was not seen with an insemination

dosage of 3 and 6×10^9spermatozoa. Semen backflow after insemination (up to 75 ml) did not affect fertilisation results. Causes of variation in backflow between sows are still poorly understood.

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2.3 Mathematical model of fertilisation

A mathematical model of conception and fertilisation in relation to insemination and ovulation in pigs

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A mathematical model of conception and fertilisation in relation to insemination and ovulation in pigs

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ABSTRACT

Fertilisation results depend on the quantity and quality of sperm cells and oocytes, and is affected by the period in which the gametes are present in the female reproductive tract. To understand a complex process like fertilisation better, mathematical models can be helpful. The objective of this study is to develop a mathematical model for fertilisation in pigs. The model should be able to demonstrate the probability of fertilisation of oocytes in pigs, dependent on the time of insemination in relation to ovulation and on the number of ovulated oocytes. In the model the process of conception (at least one oocyte fertilised in a sow) and fertilisation (proportion of oocytes fertilised) are distinguished. The parameters of the model were estimated from data of sows inseminated once with a commercial sperm dosage in 80 ml extender at various intervals relative to ovulation. The residual standard error of the final model was 0.044 and the explained variance was R^2=97%. In the model, the probability of conception is maximal 98%, when insemination is performed between 28.8 h and 3.1 h before ovulation, and the probability of good fertilisation (all oocytes fertilised) is maximal when insemination is performed at 9.6 h before ovulation. At this optimal fertilisation point, the probability of partial fertilisation is 21% and increases beyond this point. The probability of fertilisation seems to be more sensitive to timing of insemination than the probability of conception. In conclusion, the developed model gives more insight in the process of fertilisation and can be used to study effects of e.g. sperm cell and oocyte ageing on fertilisation.

INTRODUCTION

Fertilisation of oocytes is a complex process in which sperm cells and oocytes have to meet at the right time in the oviduct. The quality of oocytes (Hunter and Dziuk, 1968) and the quantity and quality of sperm cells (Hunter, 1990) are affected by the time the gametes are in the female reproductive tract until fertilisation. When

insemination takes place before ovulation the sperm cells will age, and when insemination takes place after ovulation the oocytes will age until fertilisation. Both ageing processes cause a decrease in the chances on fertilisation. This means that the moment of releasing sperm cells in the female reproduction tract (insemination) in relation to the moment of releasing the oocytes (ovulation) determines the period in which fertilisation is possible.

Elements of the fertilisation process in relation to the time of insemination and ovulation are studied extensively, wresulting in specialised knowledge about particular aspects of fertilisation (Waberski et al., 1994ab; Soede et al., 1995ab; Steverink et al., 1997). Various elements of fertilisation in relation to insemination and ovulation in the pig are combined in reviews. (Polge, 1978; Hunter, 1990; Kemp and Soede, 1997). To understand a complex process like fertilisation better, mathematical models can be very helpful. In such a mathematical model, elements have to be combined and links between different aspects of the process have to be translated into mathematical functions which can increase the insight in the process.

In literature, mathematical models have been reported that describe reproductive performance from ovulation until end of gestation as one whole process in pigs (Leymaster et al., 1986; Bennett and Leymaster, 1990ab) and in sheep (Geisler et al., 1977). In cattle, Koops et al. (1995) and Grossman et al. (1995) developed mathematical models to describe nonreturn rates for bulls from conception until end of gestation. In human, changes of fertilisation have been studied with respect to the time of coitus in relation to ovulation (Royston, 1982; Weinberg et al., 1994). In these mathematical models, special consideration has been given to a division between the process of ageing of sperm cells from ageing of oocytes. Unlike human, pig is a polytocous species and ovulate more than one oocyte. This means that besides total fertilisation of all oocytes also a part of the oocytes can be fertilised. In a study of Soede et al. (1995a) it was shown that partial fertilisation in pigs was seen in all insemination to ovulation intervals and increased when the interval between insemination and ovulation increased.

For pigs, no mathematical model of the process of fertilisation is available, although various studies have been focussed on effects of the timing of insemination on fertilisation in pigs. Such a model should give more insight in the process of fertilisation in the pig and can be used as a base for decision supporting programs for managing insemination strategies in pig production. The objective of this study

therefore is to develop a mathematical model for fertilisation in pigs. The model should be able to demonstrate the fertilisation process in pigs dependent on the time of insemination relative to ovulation and on the number of ovulated oocytes.

MATERIALS AND METHODS

Model development

The mathematical model to predict the probability of fertilisation of a number of oocytes in pigs is based on (1) the insemination to ovulation interval (Δ) and (2) the number of ovulated oocytes (N). In this study the process of conception and fertilisation are distinguished. Conception is defined as the event of at least one oocyte being fertilised after insemination (Hunter, 1967). In the case conception takes place, different levels of fertilisation are possible because one or more oocytes can be fertilised. When not all oocytes are fertilised this is called partial fertilisation. The model should be able to predict the probability of conception and the probability of fertilisation of n oocytes when Δ and N are known for a sow.

Conception rate (P_C) for one single sow is a yes or no trait. The probability of conception can be derived from the frequency of conception in a group of sows:

$$P_C = \frac{number\ of\ sows\ with\ at\ least\ one\ fertilised\ oocyte}{number\ of\ inseminated\ sows}$$

Fertilisation rate (P_F) can be calculated for one single sow as:

$$P_F = \frac{number\ of\ fertilised\ oocytes}{number\ of\ ovulated\ oocytes}$$

In case of failure to conceive, conception rate and fertilisation rate are both zero.

Conception

The probability for a sow to conceive ($P_{C,\Delta}$) is affected by the time (Δ) in hours between the moment of insemination (t_i) and ovulation (t_o), thus $\Delta = (t_i) - (t_o)$, where Δ yields negative values when insemination was applied before ovulation. $P_{C,\Delta}$ is assumed to be dependent on Δ and not on the number of ovulated oocytes N. Note that the probability of failure to conceive is $1 - P_{C,\Delta}$. The equation used for $P_{C,\Delta}$ is:

$$P_{C,\Delta} = \frac{1}{\left(1+(\frac{1}{a}-1)\times(1+e^{-(\ -d_s)})^{\frac{1}{b_s}}\times(1+e^{(\ -d_o)})^{\frac{1}{b_o}}\right)} \qquad [1]$$

where

$P_{C,\Delta}$ *is the probability of conception for an interval* Δ;

Δ *is the insemination relative to ovulation interval (h);*

d_s *is the point (h) for* Δ *where conception rate starts to decrease associated with ageing of sperm cells;*

d_o *is the point (h) for* Δ *where conception rate starts to decrease associated with ageing of oocytes;*

a *is the maximum value for* $P_{C,\Delta}$ *for the period* $d_s < \Delta < d_o$;

b_s *is an indicator for the duration (h) of decrease in conception rate associated with ageing of sperm cells;*

b_o *is an indicator for the duration (h) of decrease in conception rate associated with ageing of oocytes;*

Figure 1a shows a schematic presentation of Eq [1]. Basic assumptions made for Eq [1] are: (1) there is a range for Δ, between d_s and d_o, where conception rate is maximal, (2) the decrease in conception rate associated with ageing of sperm cells (b_s) might not be equal to the decrease associated with ageing of oocytes (b_o). The b_s and b_o indicate the duration of the decrease in conception rate from the maximum to the minimum conception rate.

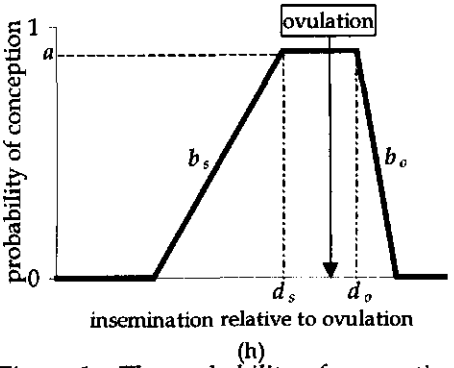

Figure 1a. The probability of conception (one or more fertilised oocytes) in relation to the time between insemination and ovulation.

Fertilisation

The probability of fertilisation (P_F) given conception is considered to be dependent on N and on Δ. First the relation of P_F with N at a fixed Δ is shown and thereafter the relation of P_F with Δ is proposed.

Given conception, a number of n out of N ovulated oocytes can be fertilised. If n is smaller than N, this is called 'partial fertilisation'. It is expected that the probability for partial fertilisation is rather constant for each n, however for n close to N an increase in probability for partial fertilisation is observed (Soede et al., 1995a).

This is translated into the following assumptions with respect to fertilisation: (1) for each n substantially different from N, there is a constant probability of fertilisation, (2) for n close to N (complete and almost complete fertilisation) the probability of fertilisation is relatively higher. Figure 1b shows a schematic pattern of these assumptions. These two assumptions give rise to applying two different distributions in the model. Assumption 1 leads to the use of an uniform distribution for describing the lower partial fertilisation and assumption 2 leads to an exponential distribution for describing complete and almost complete fertilisation. The transition point (q) separates the part in which the probability of fertilisation rate is determined by the uniform distribution from the part in which the probability is determined by the exponential distribution. For example if 20 oocytes are ovulated and q = 0.9, then the probability of less than 18 fertilised oocytes is determined by the uniform distribution, 18 fertilised oocytes is the transition point and the probability of 19 and 20 fertilised oocytes is determined by the exponential distribution. (Note that there is some small contradiction in this assumption, because in the range $q \times N$ till N there is still a small proportion which is determined by the uniform distribution. To keep the model simple we accept this small error.)

The advantage of a cumulative function is that it can be restricted and sum up to one. Therefore is the estimation of the parameters best conducted on the cumulative function in this study. Figure 1c is the cumulative

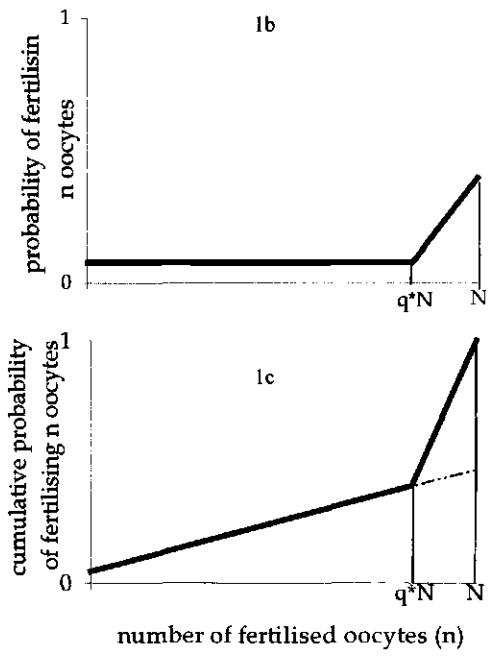

Figure 1b and 1c. The probability of fertilisation (up) and the cumulative probability (below) of n oocytes from the N ovulated oocytes in sows that have conception at a known insemination to ovulation interval, where q is the point of change from low partial fertilisation to almost complete fertilisation.

representation of Figure 1b. It shows at each n the cumulative probability of fertilisation. At $n = N$ the cumulative probability is summed to 1. The probability of complete and almost complete fertilisations (exponential) is assumed to be f, and thus the proportion of lower partial fertilisations (uniform) is equal to 1 - f. The equation used for the cumulative probability of fertilisation ($P_{F,n}$ *(cum)*) with respect to n is:

$$P_{F,n}(cum) = (1-f) \times \frac{n}{N} + f \times e^{-k \times (1-\frac{n}{N})} \qquad [2]$$

where

$P_{F,n}$*(cum) is the cumulative probability of fertilisation of n oocytes;*
N *is the total number of oocytes ovulated;*
n *is the number of oocytes fertilised;*
$1-f$ *is the probability for lower partial fertilisations (uniform sitribution);*
f *is the probability for complete or almost complete fertilisations (exponential distribution;*
k *is the transition point criteria where low partial fertilisation changes into complete or almost complete fertilisation.*

The transition point q×N is determined by parameter k in the exponential part of Eq [2]. At point q×N the probability for complete or almost complete fertilisation should be low (5% of f). This is true for the situation $e^{-k(1-q*N/N)} = 0.05$, and for $q = 1 + (1/k) \times \ln(0.05)$. For example if k is estimated as 30, the transition point is calculated as q×N = 0.90 N. From Eq [2] the probability for each n can be derived by taking the first derivative of $P_{F,n}(cum)$ with respect to n:

$$P_{F,n} = \frac{(1-f)}{N} + f \times \frac{k}{N} e^{-k \times (1-\frac{n}{N})} \qquad [3]$$

which is the mathematical representation of Figure 1b.

The model can now be extended to account for varying insemination to ovulation intervals (Δ). When ageing occurs on either sperm cells or oocytes, then the proportion of partial fertilisations will increase on the expense of the proportion of good fertilisations (Soede et al., 1995a). Therefore, an equation similar to Eq [1] can be used to make f dependent of Δ:

$$f_\Delta = \frac{1}{\left(1 + (\frac{1}{g} - 1) \times (1 + e^{-(\Delta - e_S)})^{\frac{1}{c_S}} \times (1 + e^{(\Delta - e_O)})^{\frac{1}{c_O}}\right)} \qquad [4]$$

where

f_Δ *is the probability of complete and almost complete fertilisations at interval* Δ;
Δ *is the insemination relative to ovulation interval (h);*

e_s *is the point (h) for Δ where complete and almost complete fertilisation rate starts to decrease associated with ageing of sperm cells;*

e_o *is the point (h) for Δ where complete and almost complete fertilisation rate starts to decrease associated with ageing of oocytes;*

g *is the maximum value for* f_Δ *for the period* $e_s < \Delta < e_o$;

c_s *is an indicator for the duration (h) of decrease in complete and almost complete fertilisation rate associated with ageing of sperm cells;*

c_o *is an indicator for the duration (h) of decrease in complete and almost complete fertilisation rate associated with ageing of oocytes;*

By substituting f_Δ of Eq [4] for f in Eq [3], the equation for $P_{F,n}$ is now also dependent on Δ, and the model for fertilisation rate ($P_{F,n,\Delta}$) is now complete.

Final model

The probability ($P_{\Delta,n}$) of the result of one insemination is now possible to describe at a given Δ and a given n out of N ovulated oocytes, by combining the conception rate ($P_{C,\Delta}$) and fertilisation rate ($P_{F,n,\Delta}$):

$$P_{\Delta,n} = (1 - P_{C,\Delta}) + P_{C,\Delta} \times P_{F,n,\Delta} \qquad [5]$$

For estimating the parameters it is advisable to use cumulated observations and $P_{F,n}(cum)$ instead of $P_{F,n}$ because in the cumulated model the restriction is already included that the sum of all probabilities over n is equal to 1.

Data

Data used for this study were from three experiments. Experiment 1 was designed to describe effects of moment of insemination relative to ovulation on fertilisation rate (Soede et al., 1995a). Experiment 2 was designed to study the effect of a second insemination after ovulation on fertilisation rate (Soede et al., 1995b). Experiment 3 was designed to study the effect of sperm dose at insemination on fertilisation rate (Steverink et al., 1997). From experiment 2, only data of sows that were inseminated once are used. The experimental procedures of the three experiments were similar and are described below. There were no differences between the three experiments in the effects of insemination to ovulation interval on conception and fertilisation rate.

Animals and housing

Every 2 weeks, sows were obtained at the day of weaning from a commercial farm and transported to the experimental farm. Sows were housed individually in

crates and received a total of 2.5 kg of a commercial sow diet (12.9 MJ ME kg^{-1}) in two portions daily and water *ad libitum*. Experiment 1 consisted of 143 sows, experiment 2 of 58 sows and experiment 3 of 160 sows. The number of sows from parity 1 through 8 were 49, 105, 113, 74, 10, 3, 5 and 2, respectively. The sows originated from three parental synthetic lines for commercial crossbred sows (Dalland b.v., Merselo, The Netherlands). Sows that came into oestrus and ovulated between 3 and 7 days after weaning were assigned to the study.

Ovulation

Ovulation was detected using transrectal ultrasonography as described by Soede et al. (1992). An annular array sector scanner (type 150V, Pie Medical B.V., Maastricht, The Netherlands) with a 5.0-7.5 MHz multiple scan angle transducer was used. A first check of the ovaries for presence and size of follicles (diameter of antrum > 4 mm) and corpora lutea was performed at approximately 70 h after weaning. From 16 h after the onset of oestrus, ovaries were checked at intervals of 4 h to estimate the moment of ovulation. Time of ovulation was defined as the first time when no follicles were counted minus 2 h. When the number of follicles was noticeably smaller than at the previous scanning, ovulation was assumed to have just started, since ovulation takes on average 2 h in spontaneously ovulating sows (Soede et al., 1992). Ovulation was confirmed by one additional scanning 4 h later.

Insemination

Artificial insemination was conducted once with a commercial dose of 80 ml containing 3×10^9 sperm cells in experiment 1 and 2 and 1, 3 or 6×10^9 sperm cells in experiment 3. The effect of sperm dosage was not significant on fertilisation and therefore supposed to be similar. The age of the inseminated sperm cells (time from collection to insemination) ranged from 11 to 38 h at the moment of insemination. The moment of insemination was at variable times from onset of oestrus.

Estimation of parameters

To estimate the parameters for equation [5], the results from experiment 1, 2 and 3 were pooled. The insemination to ovulation interval was divided into 10 classes of 8 h (-56 to -48, -48 to -40, -40 to -32, -32 to -24, -24 to -16, -16 to -8, -8 to 0, 0 to 8, 8 to 16 and 16 to 24). Classes -56 to -48 and 16 to 24 were excluded because of a

low number of observations (2 and 1, respectively). The probability of the fertilisation situations are calculated within each class of insemination to ovulation interval. Model parameters were estimated by nonlinear regression using adaptive nonlinear least squares algorithm (Sherrod,1994). A default value of 1×10^{-10} was used for the tolerance factor, which specifies the convergence criterion for the iterative estimation procedure. Goodness of fit of the model was measured by residual standard error.

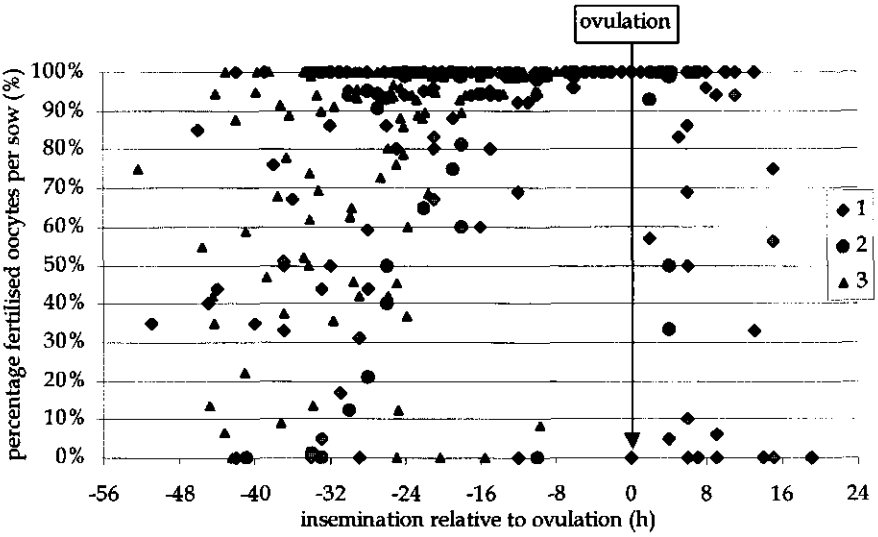

Figure 2a. Percentage of fertilised oocytes in relation to insemination to ovulation interval of 360 sows at day 5 of pregnancy from three studies: 1: Soede et al. (1995a); 2: Soede et al. (1995b); 3: Steverink et al. (1997).

RESULTS

Descriptive results

The average number of oocytes was 21 ± 3.8 (Table 1) and inseminations were performed between 52 h before ovulation and 19 h after ovulation. The percentage of fertilised oocytes varied between 0 and 100% and the variation was high (Figure 2a).

Table 1. Mean number of oocytes, insemination relative to ovulation and percentage fertilised oocytes with a SD and the range.

Variable	N	Mean	SD	min	max
Number of oocytes	360	21.0	3.8	10	36
Insemination to ovulation interval (h)	360	-18.3	14.6	-52	19
Percentage fertilised oocytes (%)	360	81.9	30.7	0	100

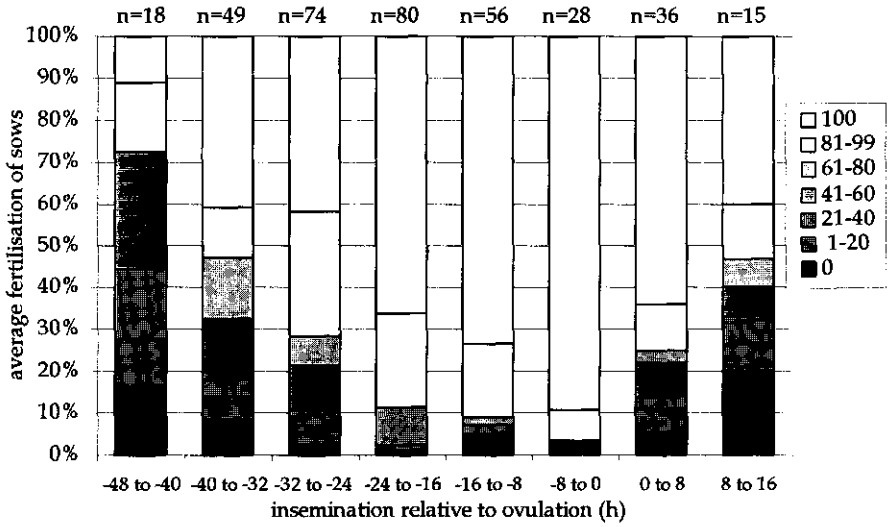

Figure 2b. The percentage of sows displaying 0%, 1-21%, 21-40%, 41-60%, 61-80%, 81-99% and 100% fertilisation rates after insemination relative to ovulation in the classes of -48 to -40 h, -40 to -32 h, -32 to -24 h, -24 to -16 h, -16 to -8 h, -8 to 0 h, 0 to 8 h and 8 to 16 h.

The highest percentage of sows with a complete fertilisation was seen when insemination was performed between 8 and 0 h before ovulation (Figure 2b). Partial fertilisation increased when insemination was performed further from ovulation with the highest number of sows with a partial fertilisation (72%) when insemination was performed between 48 to 40 h before ovulation. The proportion of sows with a lower partial fertilisation (1 to 80% fertilised oocytes) was also highest in this interval.

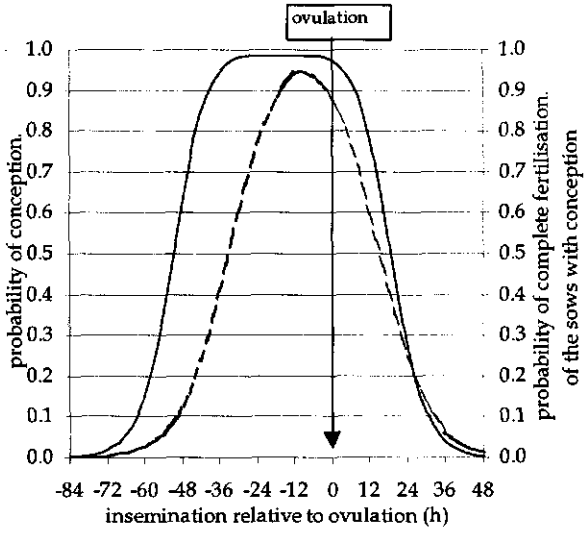

Figure 3. The estimated probability of conception (one or more fertilised oocytes) (----) and complete or almost complete fertilisation of the sows with conception (- - -) in relation to the time between insemination and ovulation.

Conception

According to the model, conception (fertilisation of at least one oocyte in a sow) was possible when insemination was performed between 84 h before and 48 h after ovulation (Figure 3). The highest probability for conception was 98.4% (a=0.984) (Table 2). The conception rate decreased due to ageing of sperm cells when insemination was performed more than 28.8 h (d_s) before ovulation. The conception rate decreased due to ageing of oocytes when insemination is performed from 3.1 h (d_o) before ovulation onwards. This point d_o was not significantly different from 0 h ($P > 0.1$). The model did not converge when the duration of the decrease in conception rate due to ageing of sperm cells (b_s) and oocytes (b_o) were estimated separately. Therefore, b_s was equated to b_o and the indicator for the duration of the decrease in conception due to ageing of sperm cells and oocytes was 5.3 h (b).

Table 2. Estimates and standard errors for the parameters used in the model to estimate probability of conception and fertilisation.

Parameter		Estimate	SE
Conception:			
	a	0.984	0.009
	d_s	-28.84	3.45
	d_o	-3.07	3.38
	b	5.29	0.73
Fertilisation:			
	g	0.958	0.010
	e	-9.56	1.35
	c_s	7.55	0.57
	c_e	7.84	1.16
	k	34.0	2.6

Fertilisation

The probability of fertilisation is illustrated by an example in which sows ovulated 20 oocytes (N=20) (Figure 4, 5 and 6). The transition point q^*N at which the uniform distribution of the lower partial fertilisation (1-f) changed into the exponential distribution of good fertilisation (f) (complete and almost complete) in a sow that ovulated 20 oocytes was n=18.3 fertilised oocytes (k=34.0) (Table 2). The probability of fertilising n oocytes (n = 1 to 20) with a given insemination to ovulation interval in sows with conception, is shown in Figure 4. The lowest probability on lower partial fertilisation (n = 1 to 18 fertilised oocytes) occurred when insemination took place between 13 and 5 h before ovulation and was in this interval 0.3%. The probability of lower partial fertilisation increased to the maximum of 5% in this example when the relative insemination to ovulation interval was lower than -48 h or higher than 29 h.

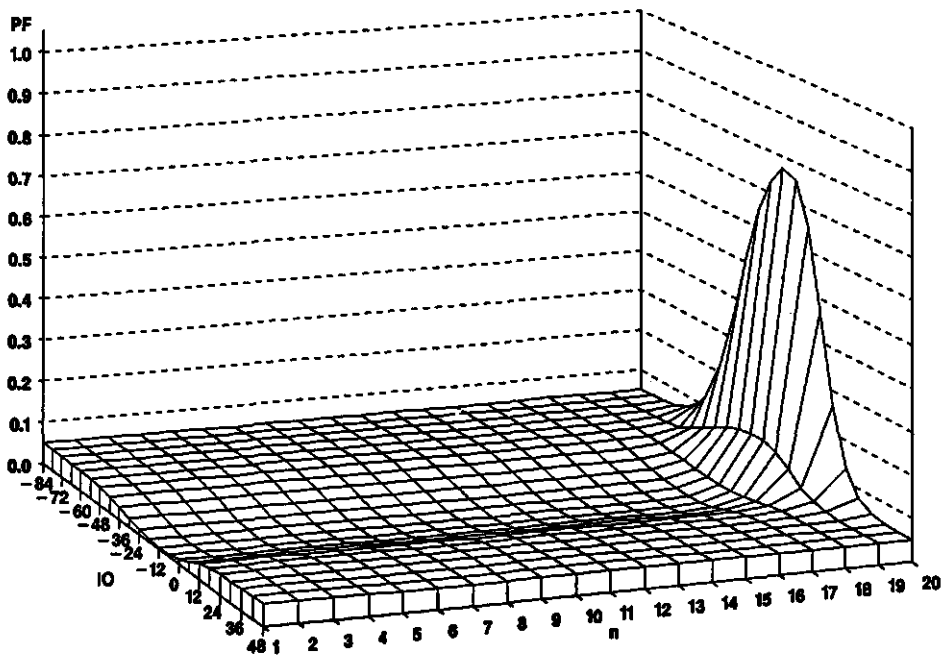

Figure 4. The estimated probability of fertilisation (PF) n oocytes in relation to insemination to ovulation interval (IO) in sows that ovulated 20 oocytes and that had conception (one or more fertilised oocytes).

The point Δ at which good fertilisation (n is 18 to 20) starts to decrease due to sperm cell ageing or oocyte ageing was similar and therefore was e_s equated to e_o to e (Table 2). The highest probability of good fertilisation (n=19 and 20) was found when insemination took place at e=9.6 h before ovulation and was 95.8% (Table 2). The indication of duration of decrease in fertilisation rate due to ageing of sperm cells (c_s) was 7.6 h and due to ageing of oocytes (c_o) was 7.8 h.

Final model

Figure 5 shows the probabilities of conception and fertilisation ($P_{\Delta,n}$) of the final model described in Eq [5], illustrated by the example in which a sow ovulated 20 oocytes. The residual standard error of the final model was 0.044 and the explained variance was R^2=97%. The probability of no conception can be read from the figure at n=0. When n is 1 to 18, the probabilities of lower partial fertilisation are shown (uniform distribution), and when n is 19 to 20 the complete and almost complete fertilisations are shown (exponential distribution).

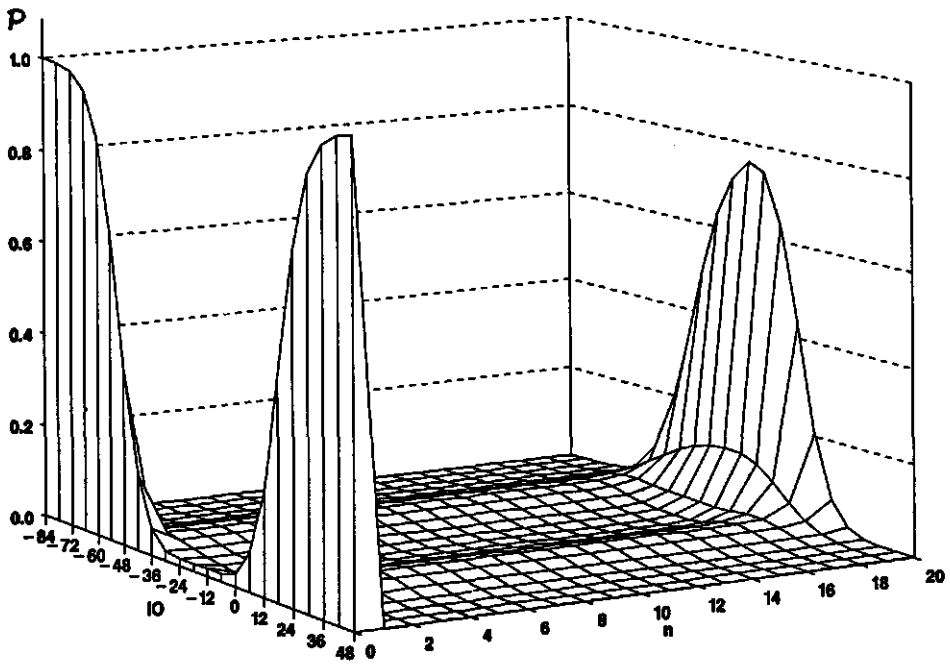

Figure 5. The estimated probability of conception and fertilisation (P) *n* oocytes in relation to insemination to ovulation interval (IO) in sows that ovulated 20 oocytes.

The highest number of fertilised oocytes can be expected between -10 and -8 h (Figure 6). On average 90% of the oocytes were fertilised when the insemination took place between 20 and 0 h before ovulation. On average 80% of the oocytes were fertilised when the insemination took place between 29 h before and 3 h after ovulation.

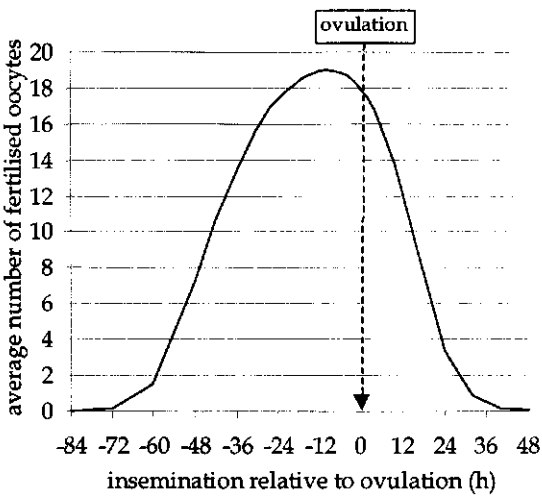

Figure 6. The average total number of fertilised oocytes in relation to the insemination to ovulation interval in sows that ovulated 20 oocytes.

DISCUSSION

General

The total number of fertilised oocytes which is determined by the number of ovulated oocytes, the conception and fertilisation rate is the starting point for the potential litter size in pigs. The mathematical model used in this study, which is a function of the time of insemination in relation to ovulation and of the number of ovulated oocytes, was able to mimic probabilities of conception and fertilisation (Figure 5) of the observed results in the 3 experiments (Figure 2) with a small residual standard error for the final model (SE=0.04).

Conception

The model suggests an interval rather then a point in which the chance of conception will be maximal. The negative effect caused by ageing of oocytes on conception already starts with insemination around ovulation. Hunter (1967) also found a decrease in conception rate immediately after ovulation. In gilts with induced ovulation, conception linearly decreased from 100 to 66.7% when insemination was applied from 0 to 16 h after ovulation. For sperm cells, it takes longer (29 h) before the effect of ageing on conception is noticeable (Figure 3). After insemination a proportion of the sperm cells reach the oviduct where a sperm reservoir is formed. Sperm cells that reach the sperm reservoir are partly protected from a reduction of motility, of viability and of fertilisation capacity (Overstreet et al., 1980; Suarez et al., 1991). Therefore, it is likely that the process of ageing is postponed while sperm cells are stored in the reservoir.

From the model it is concluded that the maximum conception rate is 98%, which means that there is always a proportion of sows that have no fertilised oocytes after insemination, even when the time between insemination and ovulation is optimal. A possible cause could be technical errors during the insemination procedure (Flowers, 1998) or ceased sperm transport. Sows normally have uterine contractions during oestrus but there is variation between pigs in frequency and amplitude of the contractions (Claus et al., 1989). In sows in which uterine contractions are absent or disturbed it is possible that too few sperm cells reach the site of fertilisation. Physical problems like uterine disorders or closed oviducts due to infection could also restrict sperm and oocyte transport (Heinonen et al., 1998).

In monotocous animals, conception rate is equal to fertilisation rate. In polytocous animals however, partial fertilisation is possible. A model on fertilisation in polytocous animals gives therefore information about differences between conception and fertilisation. This could be one of the reasons why maximum conception rate is rather high in this study (98%) compared to estimates in human (80%: Royston, 1982) and cows (76%: Grossman et al., 1995; 90%: Koops et al., 1995).

Fertilisation

In contrast to conception, fertilisation showed an optimal time point instead of an interval and this was found when insemination was applied 9.6 h before ovulation. This result indicates that negative effects on fertilisation start already with insemination 9.6 h before ovulation. Theoretically, the optimal time for insemination relative to ovulation could be the time for sperm cells to reach the site of fertilisation (Ampulla Isthmic Junction: AIJ) in sufficient numbers and capable (capacitated) of fertilising oocytes, minus the time for oocytes to reach the AIJ. The time for transportation of sperm cells from the place of insemination to the oviduct is less than 1 h (Hunter, 1984). In the oviduct, sperm cells have to move to the AIJ and capacitate before they can fertilise oocytes. Hunter et al. (1998) studied the time interval from surgical insemination into the caudal isthmus at ovulation until the time of fertilisation. When the period was 6 h it resulted in a high percentage of fertilised oocytes (46%) compared to a shorter interval (5 h: 2% fertilised oocytes). The time for transportation of sperm cells in the uterus and oviduct and thereafter capacitation should possibly be added because uncapacitated 'normal' sperm cells pass the uterotubal junction the best (Shalgi et al., 1992) and have to capacitate after they reach the oviduct. The time needed for sperm cells to reach the AIJ and be capable of fertilising will thus be 7 h. The time for the oocytes to be transported to the site of fertilisation takes less then 1 h (Hunter, 1974). Theoretically, the optimal time of insemination will thus be 6 h before ovulation (1 minus 7 h). In our model the optimal time (9.6 h) is close to this theoretical optimal time point and also within the optimal insemination to ovulation interval (0 to 24 h) as reviewed by Kemp and Soede (1997).

Final model

The distribution of conception rate and fertilisation rate were both symmetric, but the final model is asymmetric due to the mutual positioning of these distributions on the insemination to ovulation axis. In human, it was also shown that the distribution of fertilisation was asymmetric. Time taken to reduce fertilisation from 80% to 40% was 1.5 day when reduction was due to ageing of sperm cells and the time was half a day when reduction was due to ageing of oocytes (Royston et al., 1982).

From the model it is clear that the impact of partial fertilisation can not be neglected. The probability on partial fertilisation is 21% when insemination was applied at 9.6 h before ovulation and increased when insemination was performed earlier or later then this optimal moment of insemination. The probability that 1 oocyte is not fertilised or will not develop to an embryo from the total number of ovulated oocytes occurred at this interval more often (14%) than lower partial fertilisations (7%) (Figure 5). The reason that 1 or 2 oocytes are unfertilised or will not develop to an embryo after fertilisation can be for example chromosome abnormalities (McFeely, 1967) or polyspermic fertilisation (Hunter, 1967).

The insemination to ovulation interval of the studies used, ranged between -52 and 19 h. The model is extrapolated outside this range to estimate the duration of the decrease in fertilisation associated with ageing of sperm cells or oocytes. It is difficult to validate the model outside this range because no data are available. Sows are inseminated during oestrus, and oestrus duration can be up to 96 h (Weitze et al., 1994). In this extreme long oestrus duration the insemination to ovulation interval theoretically could range between -64 and 32 h with ovulation taking place at twothirds of oestrus (reviewed by Soede and Kemp, 1997). The number of sows having these extreme insemination to ovulation intervals are very rare and therefore inseminations outside the range of -52 to 19 h (our studies) will not frequently occur.

The parameters for this mathematical model are estimated from results obtained in sows that were inseminated once with a dose of 1, 3 or 6×10^9 sperm cells mixed from three boars in 80 ml. Waberski et al. (1994a) studied fertilisation results with gilts instead of sows and concluded that the optimal interval for insemination was between 0 and 12 h, but the conception rate and fertilisation rate in that interval of 0 to 12 h was still high: 100% and 92%, respectively. This interval was smaller compared to the interval of -20 to 0 h in which on average 90% of the oocytes will be

fertilised in our model. Using frozen semen in gilts decreased the optimal insemination to 0 and 4 h before ovulation in which good conception and fertilisation results (100% and 88%, respectively) could be obtained. The use of longterm-stored liquid semen (48 to 87 h and 87 to 118 h) decreased the interval from insemination to ovulation in which 'good' fertilisation results could be obtained from 0 to 24 h to 0 to 12 h compared to shorterm-stored semen (82.5% and 89.4%, respectively) (Waberski et al., 1994b). It might be speculated that if conditions for insemination are less optimal, the distribution of the model probably will be compressed and thus conception and fertilisation will be concentrated in a smaller range of insemination to ovulation interval. However, no changes in the maximum levels in the probability of conception and fertilisation are expected.

In conclusion, this model gives more insight in the process of conception and fertilisation, in the optimal moment of insemination relative to the moment of ovulation and in the effect of ageing of sperm cells and oocytes on conception and fertilisation results.

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3 Oestrus duration

3.1 Farm effect on oestrus duration

Duration of oestrus in relation to reproduction results in pigs on commercial farms

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Duration of oestrus in relation to reproduction results in pigs on commercial farms

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ABSTRACT

This research was conducted to determine factors that influence duration of oestrus, insemination strategy, and reproduction results between and within commercial swine farms that use artificial insemination. Data from 15,186 sows and gilts on 55 farms for a period of 6.1 ± 4.2 mo per farm were used in this study. The average duration of oestrus was 48.4 ± 1.0 h, ranging from 31 to 64 h, and was consistent from month to month within a farm (repeatability of 86%). Differences in duration of oestrus between farms accounted for 23% of the total variation in duration of oestrus. On most farms (n=45), gilts showed a shorter ($P<0.05$) duration of oestrus than sows (40.8 ± 1.1 h vs 48.5 ± 1.0 h). The duration of first oestrus after weaning was longer ($P<0.0001$), compared to duration of oestrus of repeat breeder sows (50.2 ± 1.0 h vs 46.8 ± 1.0 h). Duration of oestrus decreased ($P<0.05$) when interval from weaning to oestrus increased from 4 to 6 d (56.0 ± 1.2 h vs 45.8 ± 1.2 h). The regression of interval from onset to oestrus to first insemination and interval from weaning to oestrus varied between farms and ranged from -7.4 to +1.3 h/day; four farms had a positive relation. Farrowing rate decreased ($P<0.05$) when the interval from weaning to oestrus increased from 4 to 10 d (89.7 ± 2.7 h vs 78.2 ± 5.7 h). The litter size decreased ($P<0.05$) from 11.7 to 10.6 piglets when the interval from weaning to oestrus increased from 4 to 7 d. Compared with a single insemination, double insemination in sows and gilts resulted in a 4.3% and 7.0% higher ($P<0.05$) farrowing rate, respectively. When the first insemination was performed after expected ovulation, reproduction results were lower than when insemination was performed before or at expected ovulation in sows. Duration of oestrus was not related to farrowing rate or litter size in individual pigs. Number of inseminations per oestrus, time of insemination, and duration of oestrus were correlated, which made it difficult to assess which of these factors was primarily related to the farrowing rate or litter size. Knowledge of average duration of oestrus on farms and

of factors that influence the duration of oestrus on commercial farms can help to improve the efficiency of the insemination strategy specific for each farm.

Key Words: Pigs, Oestrus, Insemination, Litter Size, Reproduction

INTRODUCTION

When the interval between insemination and ovulation is between 0 and 24 h, fertilization is optimal (Kemp and Soede, 1997). Ovulation takes place approximately two-thirds of the way through oestrus (Mburu et al., 1995; Nissen et al., 1997; Steverink et al., 1997) and the duration of oestrus of sows can vary from 24 to 96 h (Weitze, 1994; Soede et al., 1995a). The duration of oestrus is influenced by factors such as parity, season, stress, boar effects, and weaning to oestrus interval (Weitze et al., 1994; Kemp and Soede, 1996; Soede and Kemp, 1997). The high variation in duration of oestrus, results in a high variation in the interval from onset of oestrus to ovulation. Therefore, onset of oestrus is not a very good predictor for the optimal time of insemination. Duration of oestrus would be a better predictor but, unfortunately, a retrospective one.

Our objectives for this study were 1) to investigate factors that influence duration of oestrus between and within commercial farms and to study whether this information can be used in a prospective way to predict duration of oestrus; 2) to study whether farms adjust their insemination strategy based on knowledge of factors affecting duration of oestrus; and 3) to investigate effects of traits related to duration of oestrus and the timing of insemination on reproduction results.

MATERIALS AND METHODS

Data and Definition of Variables

Oestrus detection was recorded on 55 commercial farms in The Netherlands from September 1989 until January 1995 by the Animal Health Service Center. A total of 15,186 records were used with an average of 279 ± 60 records per farm (60 to 749). The average number of months recorded on a farm was 6.1 ± 4.2 and ranged from 2

to 19. The average herd size was 230 pigs ranging from 90 to 630 pigs. The pigs were commercial crossbreeds that had a lactation period of 21 to 28 d.

The following variables were recorded: parity (gilt or sow) and history (repeat breeder or first insemination). Oestrus checks were performed twice daily in the presence of a boar at 08:36 h (07:12 to 10:12 h) in the morning and in the evening at 17:48 h (13:24 to 23:00 h). The farmers received instructions about the frequency and method of oestrus detection. The onset of oestrus was defined as the first time a pig showed a standing response to the back pressure test in presence of a boar, minus half the time from the former oestrus check. The end of oestrus was defined as the first time a pig did not show a standing response to the back pressure test in presence of a boar, minus half the time since the previous oestrus check. The weaning to oestrus interval was calculated in hours but expressed in days (d 1 is the first 24 h after weaning). Sows with a weaning to first oestrus interval less than 1 d (n=20) or more than 30 d (n=122) were excluded from the analyses. The date and time of each insemination was recorded (52.1% of the pigs were singly and 47.9% were doubly inseminated at one oestrus). The second insemination was performed on average 22.6 ± 0.3 h (13 to 27 h) after the first insemination. Insemination was performed with a commercial dose of pooled semen from 3 boars and containing 3×10^9 sperm cells in 80 ml and used within 2 days after collection.

Ovulation was assumed to take place at 68% of duration of oestrus (reviewed by Soede and Kemp, 1997). The interval from first and second insemination to expected time of ovulation was calculated.

Statistical Analyses

Duration of oestrus (DO), farrowing rate (FR), or litter size (LS) were analyzed with individual animal as the experimental unit (SAS, 1990). The following general model was used for analyses between groups (parity and history):

$$Y_{ijk} = \mu + F_i + P_j + H_k + \text{interactions} + e_{ijk}$$

where Y_{ijk} = a specific trait of animal; μ = overall mean; F_i = random effect of farm i (i = 1...55); P_j = fixed effect of parity j (j = 1,2); H_k = fixed effect of history k (k = 1, 2); and e_{ijk} = error term. The DO and LS were analyzed with the procedure MIXED. The FR was analyzed with the macro GLIMMIX (Littell et al., 1996), where FR was the proportion of farrowing pigs of the total number of inseminated pigs, using a logit

link function. Factors and interactions were tested for significance and omitted from the model in a stepwise way, leaving only significant factors and interactions ($P<.10$).

Farrowing rate and litter size were also analyzed within the three groups of gilts, repeat breeder sows, and weaned sows with animal as the experimental unit. The following model was used:

$$Y_{ijk} = \mu + F_i + (DO_j \text{ or } NI_j \text{ or } IO_j) + WOI_k + \text{interactions} + e_{ij}$$

where Y_{ijk} = a specific trait of animal; μ = overall mean; F_i = random effect of farm i (i = 1 to 55); (DO_j or NI_j or IO_j) = fixed effect of one of the three factors: duration of oestrus, number of insemination (NI), or interval from insemination to expected ovulation (IO). The classes of (DO_j or NI_j or IO_j) are defined as follows: DO_j = duration of oestrus of the j class (j = 1 to 5; five classes: 1 to 24, 24 to 48, 48 to 72, 72 to 96, and 96 to 120 h), NI_j = j number of inseminations (j = 1, 2), and IO_j = insemination to expected ovulation of the j class (j = 1 to 7; seven classes: 36 to 28, 28 to 20, 20 to 12, 12 to 4, 4 to –4, -4 to -12, and -12 to -20 h). These three factors (DO, NI , and IO) were correlated (r>0.5), which implies that these factors should not be included in the model at the same time. For the weaned sows, the fixed effect of weaning to oestrus interval (WOI) of the k classes was included (k = 1 to 7; seven classes: 2 to 3, 4, 5, 6, 7, 8 to 10, and 11 to 30 d).

To study the effect of farm on duration of oestrus, the variance components of the final model of duration of oestrus were used as follows: ratio of variance = $(\sigma_f^2 + \sigma_{fp}^2) / (\sigma_f^2 + \sigma_{fp}^2 + \sigma_e^2)$, where σ_f^2 = variance component of farm; σ_{fp}^2 = variance component of the interaction of farm and parity; and σ_e^2 = variance component of the error. The repeatability of the average duration of oestrus per month was calculated using the following model:

$$Y_{ij} = \mu + F_i + M_j(F)_i + e_{ij}$$

where Y_{ij} = duration of oestrus (h); μ = overall mean; F_i = random effect of farm i (i=1 to 55); M_j = random effect of the recorded calendar month j (j=1 to 3; 2nd, 3rd, and 4th mo) nested within farm; and e_{ij} = error term. The repeatability of the monthly duration of oestrus at the farms was calculated for the 2nd, 3rd, and 4th mo (the first month was not complete on most farms because farms did not start at the first day of the month) as follows: $r = \sigma_f^2 / (\sigma_f^2 + \sigma_s^2)$; where σ_f^2 = variance component of farm; and σ_s^2 = variance component of sows, calculated as $\sigma_s^2 = \sigma_m^2 + (\sigma_e^2 / n)$ where n=30 sows, which represents the average number of sows per month at the farms.

Table 1. The uncorrected average number and time of inseminations, duration of oestrus, and reproduction results, for sows, gilts, and repeat breeders across means of the farms (± SEM).

	Sows				Gilts				Sows + Gilts	
	First insemination		Repeat breeder		First insemination		Repeat breeder			
Variables	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Number of records	11,246		1,548		2,180		209		15,186	
Number of farms	55		52		53		39		55	
inseminations per oestrus, n	1.5±0.03	1.0-1.9	1.5±0.04	1.0-2.0	1.4±0.03	1.0-2.0	1.4±0.06	1.0-2.0	1.5±0.03	1.0-1.9
Duration of oestrus, h	50.1± 1.1[a]	32-69	47.5±1.1[a]	30-69	41.2±1.0[b]	19-52	40.0±2.6[b]	12-82	48.4±1.0	31-64
Oestrus to 1st insemination, h	24.3±0.7[a]	12-33	20.8±0.8[b]	8-37	18.8±0.7[c]	8-30	17.4±1.3[c]	4-38	23.1±0.6	12-32
1st SR[d] to 1st insemination, h	18.2± 0.7	6-27	14.6±0.9	3-30	12.6±0.7	2-23	10.7±0.7	0-30	17.0±0.6	6-26
Farrowing rate, %	86.9±0.7[ab]	72-95	75.4±2.0[a]	38-100	86.1±1.0[b]	63-96	81.2±4.7[a]	0-100	85.7±0.7	69-94
Litter size, n	11.4±0.1[a]	9.8-12.6	11.5±0.2[a]	8.0-13.9	9.9±0.1[b]	8.4-11.7	10.5±0.3[c]	5.0-14.0	11.2±0.1	9.5-12.3

[a,b,c] *Means within a row with different superscripts differ (P<0.05).*

[d] *SR = standing response.*

RESULTS

Descriptive Results

Of all records, 84.3% (n=12,794) were from sows and 15.7 % (n=2,389) were from gilts. Of all records, 11.5% were from repeat breeders at the time of insemination (8.7% [n=209] of the gilts and 11.9% [n=1,548] of the sows). Table 1 shows the uncorrected averages and ranges for insemination, oestrus, and reproduction traits of the farms. High variation occurs between farms for almost all traits, as reflected in the large ranges of mean values in Table 1.

Duration of Oestrus

The overall average duration of oestrus on the 55 commercial farms was 48.4 ± 1.0 h, ranging from 31 to 64 h between farms (Table 1), and was consistent over the different months within most farms. Figure 1 shows the relationship between duration of oestrus in the second (DO2) and the third recorded month (DO3). The

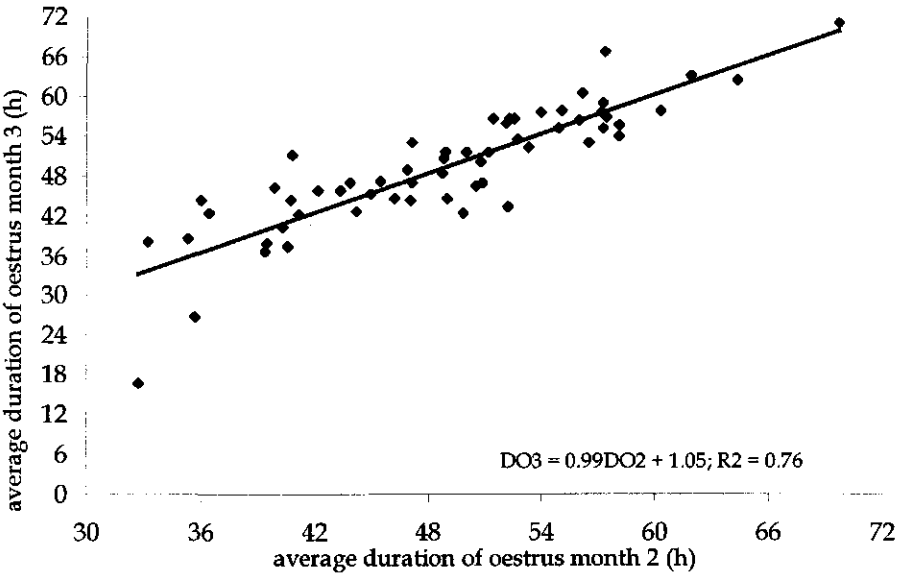

Figure 1. Relationship between the average duration of oestrus between month 2 (DO2) and month 3 (DO3) on the 55 farms.

repeatability of the monthly duration of oestrus on farms, calculated from the second, third, and fourth month was r=0.86.

On average, gilts had a shorter duration of oestrus than sows (40.8 ± 1.1 h compared to 48.5 ± 1.0 h [P<0.001]). A significant interaction between farm and parity was observed (P<0.05). On 10 of the 52 farms, there was no difference in duration of oestrus between gilts and sows. The duration of first oestrus after weaning was longer (P<0.0001) than the duration of oestrus of repeat breeder sows (50.2 ± 1.0 h and 46.8 ± 1.0 h). However, there tended to be an interaction (P=0.06) between farm and history. This means that in 9 of the 52 farms no difference was seen in duration of oestrus between first oestrus after weaning and repeat breeders. For gilts, no difference was seen in duration of oestrus of the first oestrus or repeat breeders (P=0.3). Of the total variance in duration of oestrus, 23.3% was related to the component farm (σ_f^2=37.6, σ_{fp}^2=14.0 and σ_t^2=226.9). After correction for parity,

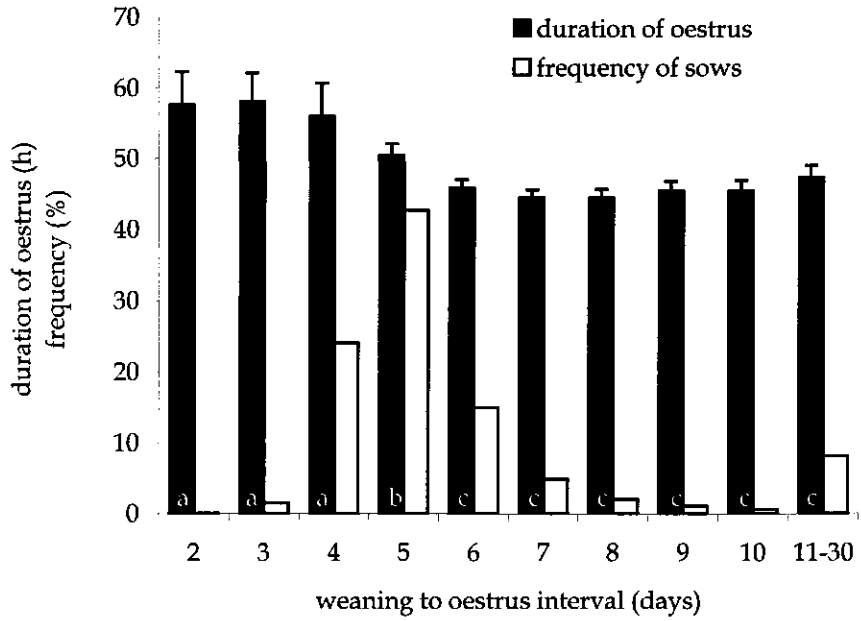

Figure 2. Distribution of sows for weaning to oestrus interval (WOI) and the average duration of oestrus for sows at the first oestrus after weaning in 10 classes of the weaning to oestrus interval (LSM ± SE).

a,b,c Different superscripts indicate significantly different duration of oestrus between WOI classes (P<0.05).

history, and farm effect (n=15,186) the duration of oestrus was 44.0 ± 1.2 h (LSM ± SEM) and ranged from 27 to 60 h between farms.

Duration of First Oestrus after Weaning

The interval from weaning to first oestrus was 5.4 ± 3.5 d (mean ± SD) and was not significantly affected by month or year. Of the sows that showed oestrus, 95% came into oestrus before 14 d after weaning; 42.7% of the sows showed oestrus on d 5 after weaning (Figure 2). On 12 farms, most sows came into oestrus on d 4 (35 to 59% of the sows at the farm), on 41 farms on d 5 (33 to 78%), on 1 farm on d 6 (36%), and on one farm a similar number of sows came into oestrus on d 5 and 6 (43%).

The duration of oestrus of all sows during their first oestrus after weaning was 50.1 ± 1.1 h (Table 1). Figure 2 shows the average duration of oestrus for 10 classes of WOI and the percentage of sows in the classes. Duration of oestrus decreased ($P<0.05$) when WOI increased from 4 to 5 d (56.0 ± 1.2 h vs 50.3 ± 1.1 h) and from 5 to 6 d (50.3 ± 1.1 h vs 45.8 ± 1.2 h). A significant interaction was observed between farm and WOI ($P<0.001$); 11 (20%) of the 54 farms did not show a significantly negative relationship between duration of oestrus and weaning to oestrus interval between d 4 and d 7.

Insemination Strategy

The first insemination of the sows was performed at 17.4 ± 1.3 h after first standing response to the boar (on average 23 h after onset of oestrus), with a range of 6 to 26 h between farms (Table 1). The average time of first insemination (**FI**) was positively related to the average DO at the farm (FI = 6.9 + 0.21 × DO; $P=0.01$; $R^2=0.11$). Gilts were inseminated sooner after first standing response than repeat breeder sows and first inseminated sows: 11.9 ± 0.7, 14.6 ± 0.6, and 18.2 ± 0.6 h, respectively ($P<0.01$). Of the total variance in time of first insemination, 27.0% was related to the component farm.

A negative relationship ($P<0.05$) was found between the interval of first standing response to first insemination and weaning to oestrus interval. When WOI increased from 4 to 7 d, insemination was earlier after first standing response (Figure 3). This negative relationship was seen in all duration of oestrus classes, except for the sows with a duration of oestrus shorter than 24 h. At the farm level, the

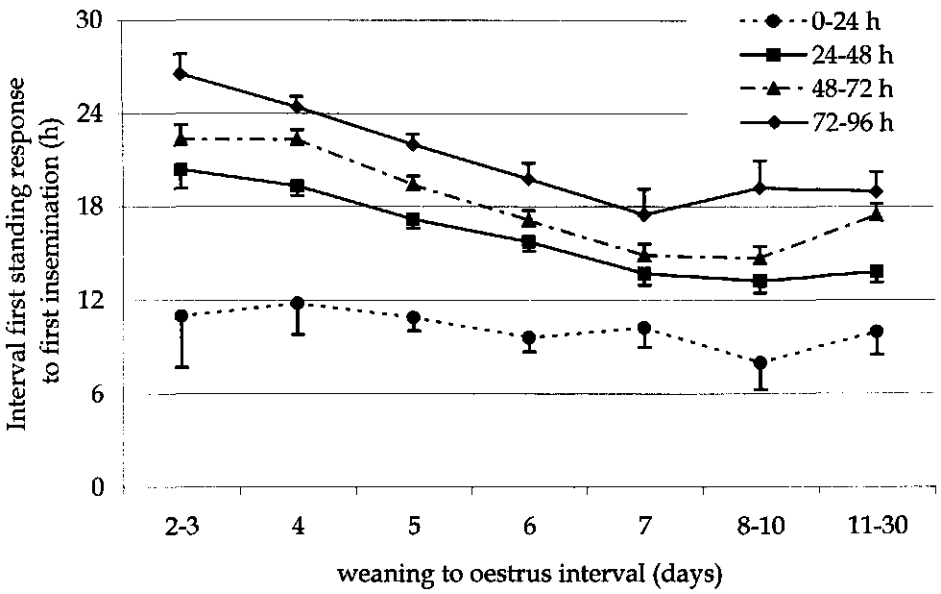

Figure 3. The timing of the first insemination in weaned sows (LSM ± SE) in relation to the first standing response to the boar in four duration of oestrus classes for seven weaning to oestrus intervals.

regression between FI and WOI ranged from -7.4 to +1.3 h/day, and four farms had a positive relationship.

Farrowing Rate

The average farrowing rate was 85.7% and ranged from 69 to 94% (Table 1) and was similar for sows and gilts ($P>0.10$). The average duration of oestrus on farms was positively correlated (r=0.29; $P=0.04$) with the farrowing rate.

The repeat breeder sows had a lower ($P<.001$) farrowing rate than sows inseminated during their first oestrus after weaning (73.2 ± 4.2 vs 85.5 ± 2.6%). The farrowing rate of the sows that were inseminated during the first oestrus after weaning was affected ($P<0.05$) by the weaning to oestrus interval, the time from first insemination to expected ovulation, and the number of inseminations. The duration of oestrus and the interactions with WOI were not related ($P>0.05$) to farrowing rate in sows. The highest farrowing rate was found when sows were first inseminated at d 4 (88.3%) or 5 (87.5%) after weaning and decreased up to d 10 thereafter (Figure 4). Sows that were inseminated twice had a 4.3% higher ($P<0.001$) farrowing rate than

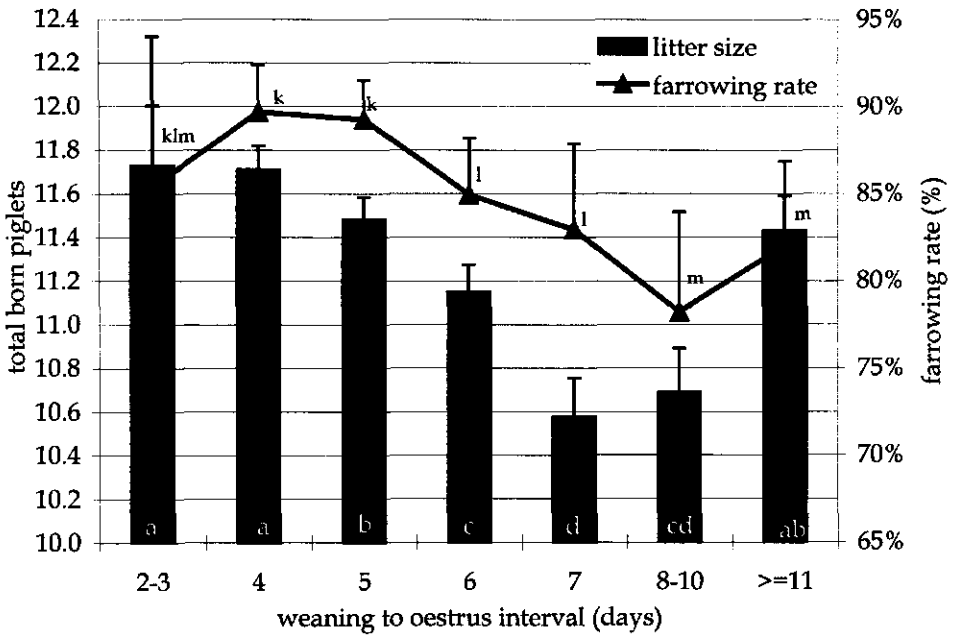

Figure 4. The farrowing rate and litter size of sows with a weaning to oestrus interval of 2 to 3, 4, 5, 6, 7, 8 to 10 and >=11 d.
[a,b,c] Different superscripts indicate significantly different litter sizes between WOI classes (P<0.05),
[k,l,m] Different superscripts indicate significantly different farrowing rates between WOI classes (P<0.05).

sows inseminated once (80.8 vs 85.1%). When a single insemination was performed, the interval from first insemination to expected ovulation resulted in the lowest farrowing rate when insemination was performed more than 4 h after expected ovulation ($LSM_{(IO=-4/-12)}$: 71.6%; $LSM_{(IO=-12/-20)}$: 59.4%; Figure 5). Doubly and singly inseminated sows showed similar trends, but this was not significant (P=0.13). However, no doubly inseminated sows were found receiving a first insemination more than 4 h after expected ovulation (Figure 5). Within the repeat breeder sows, duration of oestrus and number of inseminations did not affect farrowing rate. Insemination more than 12 h after expected ovulation tended to decrease farrowing rate.

Neither duration of oestrus nor interval from first insemination to expected ovulation (Figure 5) was related to farrowing rate (P>0.10) in gilts. However, the number of inseminations per oestrus was related to farrowing rate (P<0.05). Gilts with single or double insemination had a farrowing rate of 81.2 ± 6 and 88.2 ± 6%,

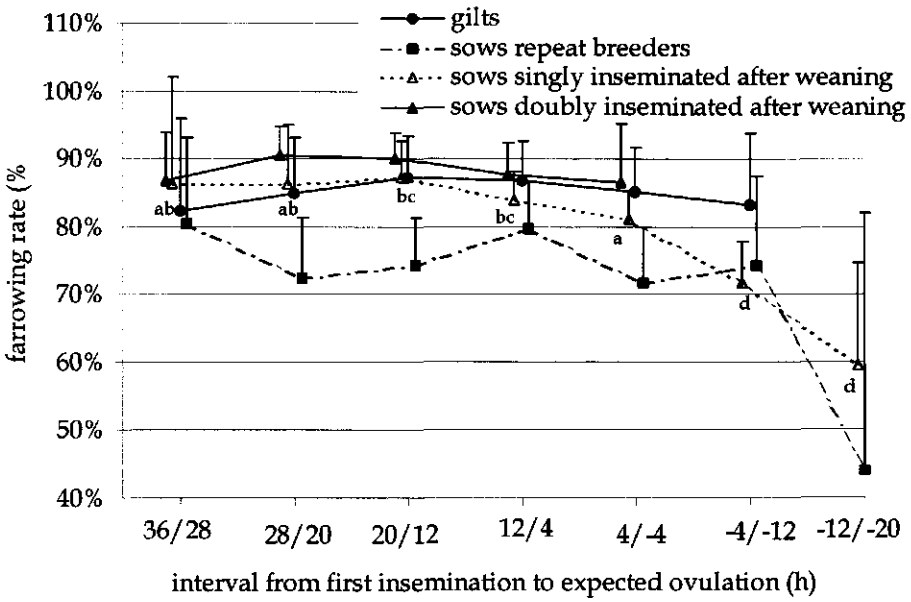

Figure 5. Farrowing rate (LSM ± SE) in seven classes of the first insemination to expected ovulation interval (IO) of gilts, repeat breeder sows, and sows at first insemination after weaning (WOI), where singly and doubly inseminated sows are separate groups.

P-value of the effect of IO on farrowing rate in gilts was P=0.7, repeat breeder sows was P=0.13, singly inseminated, weaned sows P<0.001, and doubly inseminated, weaned sows P=0.13.

a,b,c,d Different superscripts indicate significantly different litter sizes between WOI classes (P<0.05).

respectively. Repeat breeder gilts were not different (*P*=0.16) from gilts at first insemination (86.7 ± 8 vs 83.2 ± 4%).

Litter Size

The average litter size on farms ranged from 9.5 to 12.3 piglets (Table 1). The average duration of oestrus tended to be positively correlated with litter size within farm (r=0.23; *P*=0.09). The average litter size was smaller (*P*<0.01) for gilts than for sows (10.1 ± 0.2 vs 11.4 ± 0.1). Repeat breeder gilts had a higher (*P*=0.01) litter size than first inseminated gilts (10.4 ± 0.3 vs 9.7 ± 0.1) but this was not seen in sows.

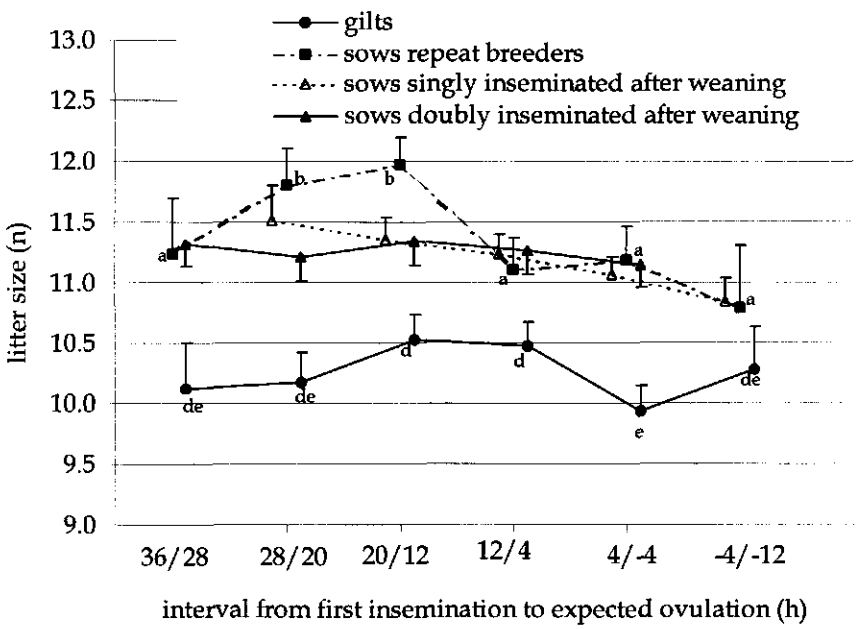

Figure 6. Litter size (LSM ± SE) in six classes of the first insemination to expected ovulation interval (IO) for gilts, repeat breeders sows, and sows at first inseminated after weaning.

The P-value of the effect of IO on litter size in gilts was P=0.06, repeat breeder sows was P=0.03, singly inseminated weaned sows P=0.09.

[a,b] Different superscripts indicate significantly different litter sizes between IO classes within repeat breeder sows (P<0.05), where total effect of IO was P=0.03.

[d,e] Different superscripts indicate significantly different litter sizes between IO classes within gilts (P<0.05) where total effect of IO was P=0.06.

The litter size of the sows that were inseminated during the first oestrus after weaning was related to the WOI. The litter size decreased ($P<0.05$) from 11.7 to 10.6 piglets when WOI increased from d 4 to 7 (Figure 4). The duration of oestrus ($P=0.2$) and the number of inseminations did not affect litter size. The interval from insemination to expected ovulation (from 36 to -12 h) tended ($P=0.09$) to have a negative relationship to litter size, decreasing from 11.5 to 10.9 piglets in the singly inseminated sows (Figure 6). In sows inseminated twice, insemination to expected ovulation interval did not influence litter size.

Within the repeat breeder sows the number of inseminations (single or double) affected ($P<0.05$) litter size (11.1 ± 0.17 vs 11.8 ± 0.16 piglets). Duration of oestrus had a positive linear relationship to litter size (LS) (LS = 10.7 + 0.017 × DO (h);

P=0.03). The largest litters were seen when first insemination was performed between 12 and 28 h before expected ovulation (Figure 6).

In gilts, the interval from first insemination to expected ovulation (IO) tended to influence litter size (P=0.07; Figure 6). The largest litters were seen when the first insemination took place between 4 and 20 h before the expected ovulation. Duration of oestrus and number of inseminations did not affect litter size in the gilts.

DISCUSSION

Duration of Oestrus

The average duration of oestrus on farms was consistent from month to month with a repeatability of 86%. Differences in duration of oestrus between farms accounted for 23% of the total variation in duration of oestrus. Because no data were collected concerning management on the farms, it is difficult to explain the origin of differences seen between farms. A possible effect could be the different interpretations of behavioral signs during oestrus detection, despite an agreement on observation method of oestrus detection. Variation between farms can also be explained by specific factors that may influence duration of oestrus: stress conditions (Soede and Kemp, 1997), differences in the quality of an individual boar (Jongman et al., 1996), breed, or, possibly, nutritional condition of sows and gilts. Overall, it is clear that the high repeatability of duration of oestrus from month to month indicates that farmers can predict the average duration of oestrus in their gilts and sows, based on information from the former month. Based on the information that ovulation takes place two-thirds of the way through oestrus (Mburu et al., 1995; Nissen et al., 1997; Steverink et al., 1997), farmers can adapt the insemination strategy based on the predicted duration of oestrus on their farm. High accuracy of oestrus detection is favorable to get a good impression of duration of oestrus. A twice daily oestrus detection program is preferable to once daily oestrus detection. When a once daily strategy is used some disadvantages can be expected. First, the duration of oestrus is less accurate, and, when time of ovulation is calculated, it will also be very inaccurate. Second, pigs with a short duration of oestrus will not be noticed to be in oestrus.

Within farms, the analyses indicated that the duration of oestrus differed between gilts and sows between first oestrus and repeat breeders, and was affected by WOI. Gilts and repeat breeders on average had a shorter duration of oestrus than sows after weaning. Parity of the sows in this study was unknown, but Steverink et al. (1997) found differences in duration of oestrus for sows in different parities. In their study, sows from parities 1 and 2 had a shorter duration of oestrus than sows from parity 3 or higher (55 vs 62 h). The effect of WOI on the duration of oestrus was seen in other studies as well (Weitze et al., 1994; Kemp and Soede, 1996; Nissen et al., 1997). However, the relationship between duration of oestrus and WOI was different between farms. The latter was also seen in a study of Flowers (1998); the frequency of sows that showed a 1, 2, or 3 d duration of oestrus on the different WOI days was different between the farms. Information about factors on a farm that affect duration of oestrus would enhance the accuracy of predicted duration of oestrus.

Insemination Strategy

The optimal time of insemination is 0 to 24 h before ovulation (Soede et al., 1995a). Ovulation takes place two-thirds of the way to through oestrus. In 1994, Weitze et al. recommended a decrease in the interval from onset of oestrus to first insemination when weaning to oestrus interval increased from 3 to 8 d, because of the decrease in duration of oestrus in this period. Because of the differences in the relationship between WOI and duration of oestrus between farms, time of insemination should be tailored to the individual farm. From the present study, one can concluded that most farms fulfill this supposition of Weitze et al. (1994) (93% of the farms). This implies that farmers use the advice in practice and might have learned from reproduction results from the past. Surprisingly, this study also showed that at each weaning to oestrus interval sows with a short duration of oestrus were inseminated sooner after first standing reflex than sows with a longer duration of oestrus. This might indicate that on these farms the timing of insemination is not only based on standing response to the boar but also on other characteristics, possibly vulva characteristics (color and mucus viscosity). Vulva characteristics (pro-oestrus) could be related to duration of oestrus; Rojkittikhun et al. (1992) showed that the duration of pro-oestrus increased and of oestrus decreased when the weaning to oestrus interval increased from 3 to 4 to 6 to 8 d. However, Sterning et al. (1994) did not find a relationship between the duration of pro-oestrus and duration of oestrus in

primiparous sows. The reason why farmers can inseminate sows according to their duration of oestrus remains unclear.

Reproduction Results

In the present study, expected ovulation time was calculated at 68% during oestrus. The calculation of expected ovulation time was done to get an impression of the effect of the interval of insemination to expected ovulation on reproduction results. The review by Soede and Kemp (1997) shows that variation in the time of ovulation in different studies ranged from 64 to 72% of oestrus, with ranges from 39 to 133% for individual sows. In this study, oestrus checks were performed only twice a day, and this might also, increase the variance for duration of oestrus. Because of this variation in the estimate of the interval from first insemination to expected ovulation (IO), this trait has to be used with caution. The calculated IO in this study should be used as an indication of the probability of insemination before ovulation, because fertilization results drop quickly when insemination is performed after ovulation (Waberski et al., 1994; Soede et al., 1995a). Therefore, negative effects on litter size and farrowing rate were expected to be found when insemination was performed after expected ovulation. This study supports this supposition, sows that had a single insemination 28 h before ovulation compared to 12 h after ovulation showed a decrease in FR from 85 to 60% and in LS from 11.5 to 10.8 piglets.

Farms that had greater farrowing rates and litter sizes also had a longer average duration of oestrus. When analyzing reproduction results with a correction for farm, duration of oestrus was not related to farrowing rates and litter sizes in any of the parity or history groups (gilts vs sows; repeat breeders vs inseminated at first oestrus after weaning). The number of inseminations, time of insemination and duration of oestrus were correlated. Therefore, it is difficult to assess which of these factors is primarily related to the farrowing rate or litter size. For instance, a second insemination was performed 24 h after the first insemination on farms when a standing response for a boar was still observed. Sows and gilts with a short duration of oestrus or with a late first insemination (close to end of oestrus) could not get a second insemination. Therefore, these pigs have a higher risk of insemination after ovulation (insemination after two-thirds of oestrus) and consequently a higher risk of suboptimal fertilization. The reproduction results were probably indirectly

influenced by duration of oestrus, via the chance to inseminate a sow or gilt at least once within the optimal period before ovulation.

Weaning to Oestrus Interval

The decrease in farrowing rate when WOI increased from 4 to 8 days was also found by Vesseur et al. (1994). Their lowest farrowing rate (58.6 ± 4%) was detected when WOI was 9 to 12 d. In this study, litter size decreased from 11.7 to 10.6 piglets when WOI increased from 4 to 7 d. A decrease in litter size when WOI increased from 4 to 10 d was seen in other studies as well (Dewey et al., 1994; Vesseur et al., 1994; Cozler Le et al., 1997). Vesseur et al. (1994) found a decrease from 11.9 to 11.1 piglets when WOI increased from 4 to 8 d. The decrease in litter size and farrowing rate with an increase in WOI was accompanied by a decrease in duration of oestrus and a decrease in the calculated time from insemination to expected ovulation. Kemp and Soede et al. (1996) found high fertilization results for sows that were inseminated between 0 and 24 h before ovulation irrespective of the WOI and duration of oestrus. Therefore, timing of insemination during oestrus relative to ovulation could be the origin for this decrease in the reproduction results. In three studies under similar conditions ovulation rate and duration of oestrus were measured (Soede et al., 1995a, b; Steverink et al., 1997). In those studies, with a total of 400 multiparous sows, ovulation rate decreased ($P<0.05$) from 21.6 to 19.7 oocytes, when weaning to oestrus interval increased from d 3 to d 6. Therefore, a decrease in ovulation rate could be a cause for the decrease in litter size with an increasing WOI that is seen in several studies.

IMPLICATIONS

Duration of oestrus and the relationship between duration of oestrus and weaning to oestrus interval differs among farms. Recording the duration of oestrus at farms for approximately a month can give an impression of the duration of oestrus for the coming period. The relationship between duration of oestrus and weaning to oestrus interval could also be studied specific for a farm. Ovulation takes place two-thirds of the way through oestrus, duration of oestrus is, therefore, the best retrospective estimate of ovulation time. The best reproduction results are depend on

the time of insemination relative to ovulation, so the insemination strategy could be optimized for each farm. Recording of the average duration of oestrus on farms and of factors that influence the duration of oestrus on commercial farms should improve the efficiency of the insemination strategy specific to each farm.

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4 PIG Simulation model for Insemination Strategies (PIGSIS)

4.1 Development of PIGSIS

Development of a simulation model to study the effect of insemination strategies on reproduction performance at commercial farms in pigs

Steverink DWB, Koops WJ, Soede NM, Noordhuizen JPTM and Kemp B

Development of a simulation model to study the effect of insemination strategies on reproduction performance at commercial farms in pigs.

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ABSTRACT

Timing of insemination can have important consequences for reproduction efficiency in pig production. The objective of this study is to develop a stochastic simulation model which can optimise and demonstrate effects of insemination strategies on farrowing rate and litter size on farms, depending on the oestrus duration of the sows. The PIG Simulation model for Insemination Strategies (PIGSIS) consists of two parts: (1) the reproduction events from the number of ovulated oocytes until the number of piglets at farrowing; (2) timing of insemination relative to ovulation based on farm parameters (weaning to oestrus interval, oestrus duration, etc.). From the farm parameters and a chosen insemination strategy, an interval between insemination and ovulation is simulated for each sow which is used to predict the conception and fertilisation rate with the first part of PIGSIS. The following physiological process are included in the model: fertilisation, embryonic mortality (due to degeneration, maternal recognition of pregnancy and uterine capacity) and foetal mortality (due to uterine capacity). From the verification and the validation it can be concluded that PIGSIS simulates reasonable reproduction results. Under the defined basic situation (oestrus duration of 47 h, average parity 4.2) and when insemination is applied between 0 to 24 h before ovulation PIGSIS simulates 12.9 total born piglets and a farrowing rate of 94.9%. In this period, embryonic, foetal and total mortality in PIGSIS was 34.9%, 3.0% and 37.9%, respectively. The number of embryos at day 1, 5 and 10 of pregnancy are clearly related to IO. After day 10 of pregnancy this effect of IO on the number of conceptuses decreased due to loss of small litters under influence of maternal recognition of pregnancy and due to increased in large litters due to embryonic and foetal uterine capacity. The effect of insemination to ovulation interval was more pronounced on farrowing rate than on litter size in PIGSIS. The farrowing rate in PIGSIS was already established at day 15 of pregnancy.

PIGSIS gives insight in the effect of inseminations strategies on reproduction results under influence of specific farm characteristics like average oestrus duration of sows and the relation between oestrus duration and weaning to oestrus interval.

Key Words: Litter size, Farrowing rate, Fertilization, Insemination Strategy, Simulation, Pigs

INTRODUCTION

The variation in farrowing rate and litter size in pigs is high and depends on many factors such as the number of ovulated oocytes, fertilisation of the oocytes and the survival of the embryos and foetuses to term. The timing of insemination relative to ovulation affects the reproductive output due to effects on fertilisation results (Soede et al., 1995a; Flowers, 1998; Nissen et al., 1997).

At commercial farms, the timing of insemination depends on the onset of oestrus. The onset of oestrus however is not a good predictor for the moment of ovulation (reviewed by Soede and Kemp, 1997). At this moment, the best retrospective predictor of ovulation is oestrus duration; ovulation takes place at on average twothirds of oestrus (reviewed by Soede and Kemp, 1997). At farms, the average oestrus duration stays at the same level during a certain period and is highly repeatable between months (Steverink et al., 1999b). There is a negative relation between oestrus duration and the weaning to oestrus interval (Kemp and Soede, 1996). Therefore, if information of the oestrus duration and the influence of weaning to oestrus interval at a farm is known, the insemination strategy could be optimised and effects on farrowing rate and litter size can be studied. The complexity of the reproduction process makes a modelling and simulation approach valuable because effects of the underlying processes and relations can be controlled by fixing or varying these parameters.

The objective of this study is to develop a stochastic simulation model which can demonstrate effects of insemination strategies on farrowing rate and litter size on farms, depending on the oestrus duration of the sows. This model should give insight in the complex physiological cascade of the reproduction results. Such a simulation model could be used for decision support program for managing insemination strategies in pig production.

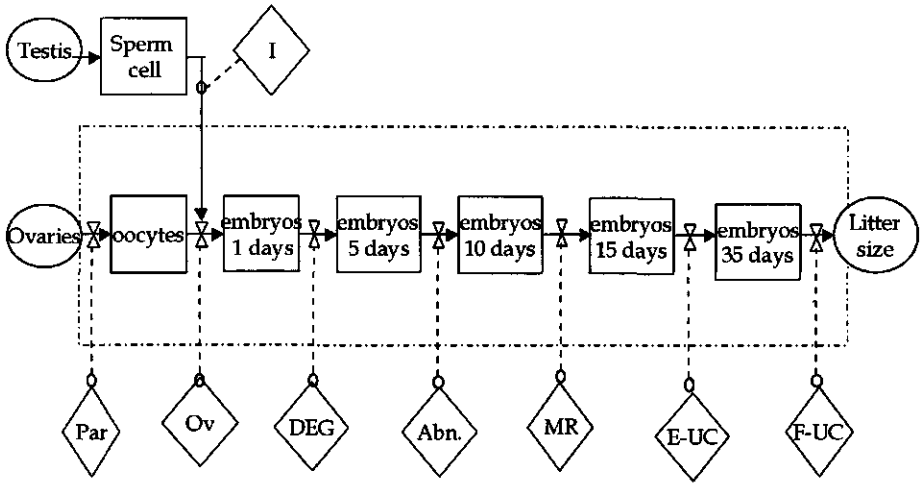

Figure 1. Flow diagram of the number of ovulated oocytes to the number of piglets at farrowing in Part I of PIGSIS.
I: Moment of insemination; Par: Parity; Ov: Moment of ovulation moment; DEG: Embryonic mortality due to degeneration: Abn: Embryonic mortality due to genetic abnormalities; MR: Maternal recognition of pregnancy; E-UC: Embryonic uterine capacity; F-UC: Foetal uterine capacity.

MODEL DESCRIPTION

Concept

Reproduction results at commercial pig farms differ in level but also in variation. To study both elements, a stochastic model is developed of the reproductive performance of sows. The PIG Simulation model for Insemination Strategies (PIGSIS) consists of two parts. The first part simulates the reproduction events from the number of ovulated oocytes until the number of piglets at farrowing. In the second part of PIGSIS the farm parameters (weaning to oestrus interval, oestrus duration, etc.) and insemination strategies are introduced. From these farm parameters an interval between insemination and ovulation will be simulated for each sow which will be used to predict the conception and fertilisation rate in the first part of PIGSIS. PIGSIS is programmed in SAS (1997).

Part I: Number of ovulated oocytes until farrowing rate and litter size

In the simulation model, five intermediate moments of the pregnancy stage

are used: day 1, 5, 10, 15 and 35 (Figure 1). The processes that are responsible for the effects between stages are: conception and fertilisation (day 1), degeneration of embryos (day 5), genetic abnormalities of embryos (day 10), maternal recognition of pregnancy (day 15), embryonic uterine capacity (day35) and foetal uterine capacity (day 110).

1. The first step in PIGSIS generates a number of oocytes at ovulation for each sow. The number of ovulated oocytes (OO) is assumed to be normally distributed and depends on the parity (Par) of the sow:

$$OO = 23.1 - \frac{5.08}{Par} + e_{oo} \quad [1]$$

where
$e_{oo} \approx N(0, 3.7)$ *oocytes, which represents the residual standard deviation;*
OO is rounded to the nearest integer value of ovulation rate;
Par is the parity of the sows (1, 2,..., 11; where 1 is first litter sow)
$R^2 = 0.97$; $P < 0.001$ (Soede et al., 1995ab; Steverink et al., 1997).

2. The number of embryos at day 1 (E1) depends on the number of ovulated oocytes and on the probability of fertilisation (FR) of those oocytes. Steverink et al. (1999a) described a mathematical model for the probability of conception and fertilisation in pigs depending on the moment of insemination in relation to ovulation (IO) and on the number of ovulated oocytes in a sow. In this model the effects of ageing of sperm cells (insemination before ovulation) and ageing of oocytes (insemination after ovulation) are estimated. For each sow the probability of conception (at least one oocyte fertilised) and the probability of fertilisation (partial and complete: 1, 2, ..., OO embryos) given conception is calculated resulting in a discrete distribution for a sow with OO (for example OO=20; Figure 2 from Steverink et al., 1999a)

$$E1 = OO \times P_{FR} \quad [2]$$

where
P_{FR} *is the discrete probability for each sow to have 0, 1,..., OO embryos and depends on the moment of insemination in relation to ovulation and on the number of ovulated oocytes (Steverink et al., 1999a).*

3. Between day 1 and 5 of pregnancy some embryos degenerate (Soede et al., 1995a). The probability of sows to have degenerated embryos (SowD) given conception,

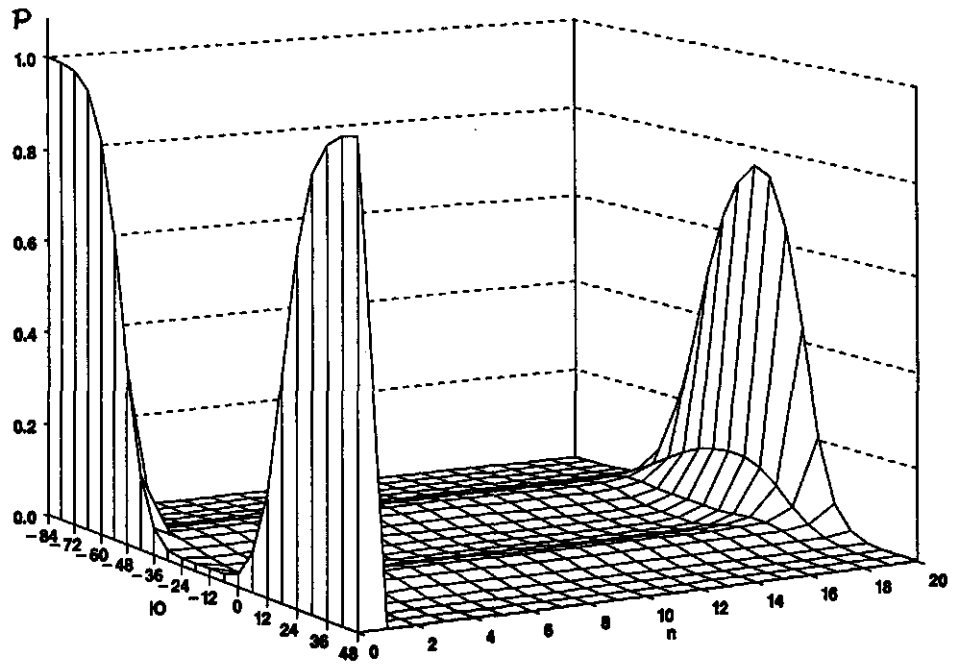

Figure 2. The estimated probability (P) of conception (at least 1 oocyte fertilized) and fertilization of n oocytes in relation to insemination to ovulation interval (IO) in sows that ovulated in this example 20 oocytes (Steverink et al., 1999a)

was related to the fertilisation rate of the sow as follows:

$$P_{SowD} = 0.868 - 0.639 \times \left(\frac{E1}{OO}\right) \qquad [3a]$$

where
P_{SowD} is the binomial probability of a sow having at least one degenerated embryo given conception (range P_{SowD} 0.229 to 0.868);
P <0.001; -2LOGL=33.7; (Soede et al., 1995ab; Steverink et al., 1997).

The number of degenerated embryos of a sow that was assigned to have degenerated embryos [3a] is related to the fertilisation rate of the sow. The probability of embryos to degenerate (P_{DEG}) given presence of degenerated embryos is:

$$P_{DEG} = 0.843 - 0.788 \times \left(\frac{E1}{OO}\right) \qquad [3b]$$

where
P_{DEG} *is the probability of an embryo to degenerate,* given presence of degenerated *embryos (range* P_{DEG} *0.843 to 0.055);*
R^2 *=0.60;* $P < 0.001$ *(Soede et al., 1995ab; Steverink et al., 1997).*

The number of embryos at day 5 (E5) of pregnancy depends on the P_{SowD}, P_{DEG} and the number of embryos at day 1 of pregnancy:

$$E_5 = E1 \times (1 - P_{SowD} \times P_{DEG}) \quad [3c]$$

where
P_{sowD} *is proposed by equation [3a] and* P_{DEG} *is proposed by equation [3b].*

4. Little is known about embryo mortality between day 6 and 10 of pregnancy. One of the causes are genetic abnormalities (Hafez, 1993). McFeely (1967) found that on average 10% of the embryos had chromosomal abnormalities, that usually resulted in death of the embryo. The proposed probability of an embryo to die is generated by a binomial distribution with an average probability of mortality of 10% (PM_{6-10}), after which the number of embryos at day 10 (E10) is:

$$E10 = E5 \times (1 - PM_{6-10}) \quad [4]$$

where
PM_{6-10} *is the binomial probability of an embryo to die.*

5. The number of embryos at day 15 (E15) of pregnancy is depends on the maternal recognition of pregnancy (MR). When 4 or less embryos are present in the uterus there is no maternal recognition of pregnancy (Polge et al., 1966). When 5 embryos are present at least 2 embryos in each uterine horn are necessary for maternal recognition (Polge et al., 1966). Spacing of the embryos is supposed to be a random process in which embryos can be in the right or left uterine horn (drawing without replacement). When 5 embryos are present in the uterus the chance is 62.5% that in both uterine horns at least two embryos are present. When 6 embryos are present in the uterus the chance is 78.1%. If 7 or more embryos are present in the uterus, it is supposed that there always will be maternal recognition of pregnancy (Polge et al., 1966).

$$E15 = E10 \times PS_{MR} \quad [5]$$

where
$E10 \leq 4$ *then* $PS_{MR} = 0.0$;
$E10 = 5$ *or* 6 *then* $PS_{MR} = 1\text{-}2\times[.5^{E10}+E10\times.5^{E10})$;
$E10 \geq 7$ *then* $PS_{MR}=1.0$.

6. The uterus has a limited capacity for embryos to survive which is called in this study embryonic uterine capacity. The number of embryos at 35 days (E35) of pregnancy depends on the probability of embryonic survival between day 16 and 35 (PS_{16-35}). It is assumed that there is a negative correlation between E15 and survival. The relation is supposed to be independent to the parity of sows. The relation is estimated from a study with first parity sows (R^2=0.52; P<0.001; Van den Brand, unpublished results). The probability of survival of embryos in a sow up to day 35 will be:

$$PS_{16-35} = \frac{31.43}{E15} \times (1 - e^{\left(\frac{-E15}{31.43}\right)} \quad [6]$$

where
PS_{16-35} is the binomial probability of an embryo to survive between day 16 and 35 of pregnancy due to embryonic uterine capacity;
E15 is number of embryos at day 15 of pregnancy;
R^2=0.52; P<0.001 (Van den Brand, unpublished results).

The number of embryos at day 35 of pregnancy (E35) is:

$$E35 = E15 \times PS_{16-35} \quad [7]$$

7. The number of piglets at farrowing is limited by the capacity of the uterus to carry foetuses to term. The maximum number of piglets for a sow is based on the foetal uterine capacity (UC) (Leymaster et al., 1986; Wu et al, 1987). The foetal uterine capacity is the average upper limit of the number of foetuses that can survive. A linear-plateau model is used for the distribution of the probability of survival of foetuses between day 36 and 110:

$$PS_{36-110} = \left(\frac{UC - ln(1 + e^{-1.013 \times (E35 - UC)}}{E35} \right) \quad [8]$$

where
PS_{36-110} is the binomial probability for each foetus to survive between day 36 of pregnancy and farrowing due to foetal uterine capacity;
E35 is the number of embryos at day 35 of pregnancy;
UC is foetal uterine capacity of the sow.

The average uterine capacity depends on the parity of a sow and is arbitrarily chosen: 14.0, 15.0, 15.5 and 16.0 piglets in parity 1, 2, 3 and 4 to 11 sows, respectively. The total number of piglets born at farrowing (E110) is:

$$E110 = E35 \times (PS_{36\text{-}110}) \quad [9]$$

8. Litter size in PIGSIS is defined as the total number of born piglets (death and live). Farrowing rate is the percentage of sows that give birth to at least 1 piglet in relation to the total number of inseminated sows. (Note: Hardly any termination of pregnancies after day 15 occur, since late pregnancy failure (abortions, pseudopregnancy, etc.) are not taken into account in PIGSIS).

Part II: The events of weaning until end of oestrus

Figure 3 shows a number of events that are related to farm and management effects used in PIGSIS. The farm parameters (weaning to oestrus interval, oestrus duration, parities of the sows) can be considered as input parameters for the simulation model. In this paragraph a standard value is suggested for the parameters which can be seen as an average farm in the Netherlands and is defined as the basic situation.

The parity of the sows in PIGSIS is a reflection of an average Dutch herd (SIVA, 1998) but gilts were excluded. Sows are generated with a parity from 1 to 11 following a discrete distribution with probabilities of: 0.20, 0.15, 0.14, 0.13, 0.09, 0.08, 0.06, 0.05, 0.04, 0.03 and 0.03, respectively.

Weaning is the moment of the start of the cycle (8.00 in the morning). The first 24 h after weaning is defined as day 1 after weaning. The distribution of the weaning to oestrus interval (WOI) for sows is a mixture of a normal N(5.13, 1.00) and an exponential distribution E(λ=5.00) (Ten Napel et al., 1995). The normal distribution represents sows with a normal WOI (1 until 7 days) and the exponential distribution

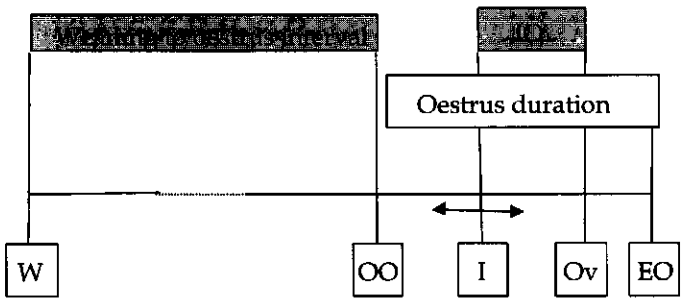

Figure 3. Time schedule of weaning, oestrus and insemination events in Part II of PIGSIS.
W: moment of weaning; OO: Onset of oestrus; I: moment of Insemination; Ov: Moment of ovulation; EO: End of oestrus; IO: Insemination to ovulation interval.

represents sows with a prolonged WOI (more than 7 days). Parity 1 sows have a higher chance for a prolonged WOI compared to older sows and therefore a higher proportion of sows are part of the exponential distribution rather than the normal distribution. The proportion of sows in the normal and the exponential distribution is assumed to be: 0.7 and 0.3 for parity 1 (Ten Napel et al., 1995); 0.85 and 0.15 for parity 2; and 0.9 and 0.1 for parity 3 and older, respectively.

The oestrus duration in PIGSIS is the total time that a sow showed a standing response to a boar. Oestrus duration (OD) is negatively related to WOI (Kemp and Soede, 1996). The average oestrus duration in their two experiments was 53 h and 59 h. The regression in this linear relation between OD and WOI was similar for both experiments (R^2=0.25; P>.001). The proposed linear relation of OD and WOI in PIGSIS for sows with a WOI less then 144 h is:

$$OD = 88 - 0.33 \times WOI + e_{OD} \quad [10]$$

where
WOI is expressed in hours;
$e_{OD} \approx N(0, 10\ h)$, which represents the residual standard deviation of OD;
Kemp and Soede (1996).

When WOI is 144 h or more the oestrus duration remains at a level of this WOI. In this basic situation this is 37 h.

Ovulation (Ov) takes place at on average twothirds of the way through oestrus which is found in a number of studies; 68% (Mburu et. al., 1995); 72% (Soede et al., 1995a); and 68% (Steverink et al., 1997). From the 3 studies: Soede et al. (1995ab) and Steverink et al. (1997) it could be seen that sows with a short oestrus duration (<24 h) had on average an ovulation closer to the end of oestrus. The proposed normal distribution (with SD = 0.10) of the relative moment of ovulation (RO) in relation to oestrus duration is therefore:

$$RO = 0.50 + \left(\frac{10.06}{OD}\right) + e_{RO} \quad [11]$$

where
OD is expressed in hours;
$e_{RO} \approx N(0, 0.10)$, which represents the residual standard deviation of RO;
R^2=0.98; P<0.001 (Soede et al., 1995ab; Steverink et al., 1997).

The insemination to ovulation interval (IO) is defined as the moment of insemination minus the moment of ovulation (Figure 3). The insemination strategy applied consisted of one insemination (I) (containing 3×10^9 sperm cells in 80 ml BTS-

extender of good quality used within 48 h after collection) for each sow, 24 h after the onset of oestrus as detected by the farmer. The oestrus detection strategy used in PIGSIS is performed twice a day at 8.00 h and 18.00 h by using a boar.

Sensitivity analyses

The relevance of the developed model is partly determined by its behaviour when the input variables are varied. Sensitivity analyses are carried out by varying the values of 6 important parameters. These parameters are chosen because they show the most likely effects on the reproduction events in relation to insemination strategies. The parameters are presented in Table 1. For each parameter (except for embryonic uterine capacity and oestrus detection strategy) 5 scenarios are studied: the basic value as described in the material and method section, the basic value $\pm \sigma$ and the basic value $\pm 2\sigma$. Each scenario consists of 10,000 sows; such a large number has been chosen to avoid disturbances due to small samples. For example, the 5 scenarios for the number of ovulated oocytes result in the following values for a parity 3 sow: 14.0, 17.7, 21.4, 25.1 and 28.8 oocytes. The embryonic survival between day 16 and 35 ($ES_{16\text{-}35}$) of pregnancy is varied by changing the upper limit in the exponential distribution for embryonic uterine capacity (basic limit is 31.4 ± 2.2). For the scenarios of the embryonic survival between day 16 and 35 a larger contrast is chosen because most litters are below the limit of 31.4 embryos. The scenarios will therefore be 2 and 4 times SE deviated from the basic upper limit (22.6, 27.0, 31.4, 35.8

Table 1. Levels of the scenarios of the population parameters and farm and management parameters used in the sensitivity analyses.

Parameters	N	Levels[1]
Population parameters:		
Oocytes	10000	μ-7.4, μ-3.7, μ, μ+3.7, μ+7.4
Embryonic uterine capacity (d15 to 35)[2]	10000	22.6, 27.0, 31.4, 35.8, 40.2
Foetal uterine capacity (d36 to term)	10000	μ-4, μ-2, μ, μ+2, μ+4
Farm and management parameters:		
Oestrus duration (h)	10000	27, 37, 47, 57, 67
Oestrus detection strategy (times daily)	10000	1, 2, 3
Insemination strategy (h)	10000	0, 12, 24, 36, 48

[1] *μ refers to the mean of the parameter for the parity in the basic situation;*
[2] *levels are created by changing the upper limit of the embryonic survival in the basic situation.*

and 40.2). For the foetal uterine capacity the standard deviation is supposed to be 2 piglets. The 5 scenarios for uterine capacity of a sow of parity 3 are: 11.5, 13.5, 15.5, 17.5 and 19.5. Three farm and management parameters are varied: the average oestrus duration (27, 37, 47, 57 and 67); oestrus detection strategy by once (0800), twice (0800 and 1800) or 3 times (0800, 1600 and 2400) a day; and insemination strategy were one insemination is applied at either 0, 12, 24, 36 or 48 h after the detected onset of oestrus.

Validation

The level of embryonic and foetal mortality are validated with data from Van der Lende and Schoenmaker (review; 1990), Lambert et al. (1991) and Pere et al. (1997).

The reproduction results (part 1 of PIGSIS), events from the number of ovulated oocytes until farrowing rate and litter size in relation to the insemination to ovulation interval, are validated with a study of Nissen et al. (1997). They studied the

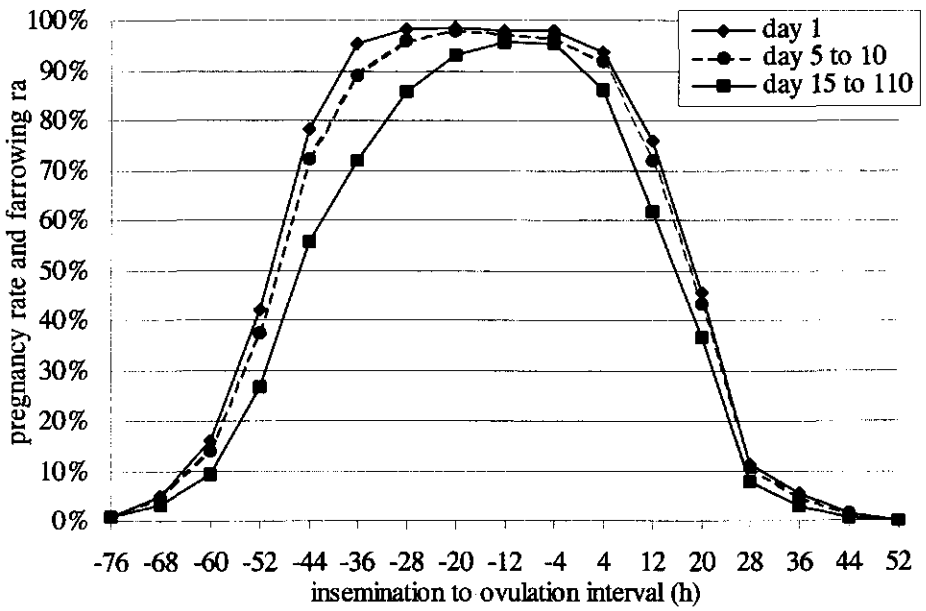

Figure 4. Farrowing rate at day 1, 5 (and 10), and 15 to 110 in relation to the insemination to ovulation interval as simulated by PIGSIS for the basic situation.

effect of IO on E10 (n=20), farrowing rate (n=91) and litter size (n=75). 10,000 Sows are sampled with the parameters of the basic situation as described in the material and method section and the outcome is compared with results of Nissen et al. (1997). The average number of oocytes of PIGSIS is adjusted (basic +1.0 oocytes) to the number of oocytes found in Nissen et al (1997).

RESULTS

Basic situation

The basic situation as described in the material and method section is used to show the results of PIGSIS. The percentage of sows with conception (at least one embryo) was more than 90% when insemination was applied between 38 before and 5 h after ovulation (Figure 4). The average number of pregnant sows decreased two times: from day 1 to 10 and from day 10 to 15. The latter had the highest impact on terminating pregnancy of those two and was caused by maternal recognition. The decrease of number of pregnant sows between day 10 and 15 was more than 10 %, when insemination was applied more than 28 h before and more than 10 h after ovulation. The highest simulated average farrowing rate is 95.8% when insemination is applied between 8 and 16 h before ovulation. The farrowing rate was higher than 90% when insemination was applied between 0 and 25 h before ovulation. Figure 5 shows the average number of embryos at several stages of pregnancy as affected by the insemination to ovulation interval (IO). The differences between the lines represent the loss of number of embryos or foetuses between stages of pregnancy. The average number of oocytes was 21 irrespective of the interval between insemination and ovulation. Early embryonic mortality of the pregnant sows (day 0 to 10) was on average 17% when insemination was applied between 0 and 24 h before ovulation from which 7% was due to fertilisation of the sows that were still pregnant. Total embryonic mortality of the pregnant sows (day 0 to 35) was 35% in this IO of the pregnant sows at d 35. Total embryonic and foetal mortality was 38% of the sows that farrowed that were inseminated between 24 and 0 h before ovulation. There is a pronounced effect of insemination to ovulation interval on the number of embryos at day 1 until day 10 of pregnancy. This effect of IO on the average number of embryos becomes less pronounced due to loss of the small litters (maternal

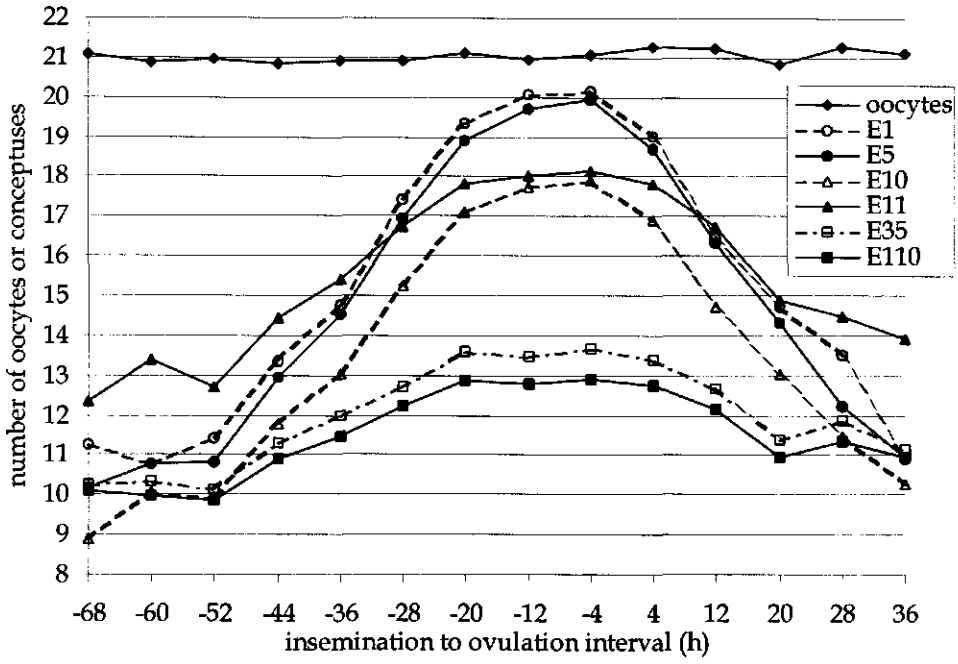

Figure 5. Number of oocytes, embryos (day 1, 5, 10, and 15) and foetuses (day 35 and 110) in relation to the insemination to ovulation interval as simulated by PIGSIS for the basic situation.

recognition) around day 14 of pregnancy. The increase of the average number of embryos from day 10 to day 15 was due to this loss of the small litters. The effect of IO decreased further due to embryonic mortality up to d 35 and due to foetal mortality both caused by the uterine capacity. The limiting effect of the embryonic uterine capacity caused selective mortality of embryos in large litters. Therefore, the effect of insemination to ovulation interval is less pronounced on litter size at farrowing than at number of conceptuses at earlier stages of pregnancy.

Sensitivity for the number of oocytes

A decrease of on average 7.4 oocytes resulted in a decrease of 4.4% in farrowing rate and a decrease in litter size of 3.1 piglets (Table 2). An increase of 7.4 oocytes increased farrowing rate with 0.8% and the number of piglets with 1.5. A decrease of the number of ovulated oocytes has a larger impact on reproduction results than an increase in the number of oocytes compared to the basic situation.

Table 2. The simulated average and SE of the number of oocytes, farrowing rate and litter size of the basic situation and the deviation from the basic situation for the proposed scenario's of number of oocytes (-2, -1, 1 and 2)[1], when insemination is applied between 24 and 0 h before ovulation (n=1855 per scenario).

	-2	-1	basic[2]	1	2	SE
Oocytes	-7.4	-3.8	21.0	+3.7	+7.4	
Farrowing rate (%)	-4.4	-0.7	93.7	+0.5	+0.8	0.60
Litter size	-3.1	-1.4	12.9	+1.0	+1.5	0.05

[1] *-2: basic-2σ; -1: basic-1σ; 1: basic+1σ; 2: basic+2σ;*
[2]*basic refers to the basic situation described in the material and method section.*

Sensitivity for the embryonic uterine capacity

The different scenarios for embryonic survival between day 11 and 35 of pregnancy did not influence farrowing rate. Only sows with large litters are negatively affected by an increased embryonic mortality but this did not result in termination of pregnancy. A decrease in the upper limit for embryonic uterine capacity with on average 8.8 embryos resulted in a decrease of 1.0 piglets (Table 3), whereas an increase of the upper limit with 8.8 embryos increased litter size with 0.5 piglets. A decrease of the upper limit for embryonic uterine capacity has higher impact on litter size than an increase compared to the basic situation.

Table 3. The upper limit of embryonic uterine capacity at day 35 of pregnancy used in the scenarios and the simulated average and SE of farrowing rate and litter size of the basic situation and the deviation from the basic situation for the proposed scenario's of foetal uterine capacity (-2, -1, 1 and 2)[1], when insemination is applied between 24 and 0 h before ovulation (n=1922 per scenario).

	-2	-1	basic[2]	1	2	SE
Upper limit	22.6	27.0	31.4	35.8	40.2	
Farrowing rate (%)	-0.0	-0.0	93.9	+0.0	+0.0	0.52
Litter size	-1.0	-0.4	12.9	+0.3	+0.5	0.06

[1] *-2: basic-2×SE; -1: basic-2×SE; 1: basic+2×SE; 2: basic+4×SE;*
[2]*basic refers to the basic situation where the upper limit is 31.4 piglets described in the material and method section.*

Sensitivity for the foetal uterine capacity

The scenarios for foetal uterine capacity did not influence farrowing rate. Sows with large litters are negatively affected by uterine capacity but this did not result in termination of pregnancy. A decrease of the uterine capacity with on average 4 foetuses resulted in a decrease of 2.0 piglets at farrowing (Table 4). An

increase of the foetal uterine capacity with 4 foetuses increased liter size with 0.6 piglets. A decrease of the uterine capacity has higher impact on reproduction results than an increase compared to the basic situation.

Table 4. The foetal uterine capacity used in the scenarios and the simulated average and SE farrowing rate and litter size of the basic situation and the deviation from the basic situation for the proposed scenario's of embryonic uterine capacity (-2, -1, 1 and 2)[1], when insemination is applied between 24 and 0 h before ovulation (n=1865 per scenario).

	-2	-1	basic[2]	1	2	SE
Uterine capacity	11.4	13.4	15.4	17.4	19.4	
Farrowing rate (%)	-0.0	-0.0	94.6	+0.0	+0.0	0.52
Litter size	-2.0	-0.8	12.8	+0.4	+0.6	0.05

[1] *–2: basic-2σ; –1: basic-1σ; 1: basic+1σ; 2: basic+2σ.*
[2]*basic refers to the basic situation described in the material and method section.*

Sensitivity for oestrus duration

The average oestrus duration was increased with 10 h in each scenario, which resulted in a decrease of the average insemination to ovulation interval of 5 h (Table 5). The effect of oestrus duration on farrowing rate (range 88.4% to 93.5%) is larger than on litter size (range 12.6 to 12.8 piglets). The result of one insemination at 24 h after detected onset of oestrus (expressed in total piglets per insemination) was highest for the basic scenario (12.0 piglets per insemination). Either an increase or a decrease of oestrus duration compared to the basic situation has similar negative effect on the reproduction results.

Table 5. The average and SE of the simulated oestrus duration, insemination to ovulation interval (IO), farrowing rate and litter size of the basic situation and the deviation from the basic situation for the proposed scenario's of oestrus duration (-2, -1, 1 and 2)[1], when insemination is applied 24 h after detected onset of oestrus.

	-2	-1	basic[2]	1	2	SE
Oestrus duration (h)	-20.0	-10.0	47.7	+10.0	+20.0	0.12
IO (h)	-9.9	-5.0	-9.8	+5.0	+10.1	0.1
Farrowing rate (%)	-5.1	-1.4	93.5	-1.2	-4.1	0.3
Litter size	-0.1	-0.0	12.8	-0.0	-0.2	0.05

[1] *–2: basic-2σ; –1: basic-1σ; 1: basic+1σ; 2: basic+2σ.*
[2]*basic refers to the basic situation described in the material and method section.*

Sensitivity for oestrus detection strategy

The variation (SD) in oestrus duration decreased from 15.8 to 12.8 when the frequency of oestrus detection increased from 1 to 3 times daily (Table 6). In this simulation, with on average an oestrus duration of 46.9 h, 55 sows were not detected in oestrus with a once daily detection strategy, whereas 2 and 3 times daily oestrus detection did not detect 3 and 2 sows, respectively. The number of sows inseminated in the optimal IO decreased from 83.5% to 71.5% when oestrus detection strategy changed from a 3 times daily oestrus detection to a once daily detection. This decrease in detection frequency (from 3 to 1) results in a small negative effect of 2.3% in farrowing rate and 0.3 piglets at farrowing.

Table 6. The average oestrus duration, SD of oestrus duration, frequency of sows in the optimal insemination to ovulation interval (IO) (-24 h to 0 h), farrowing rate and litter size of the simulated real oestrus duration of the 3 scenarios of oestrus detection frequency (1, 2 and 3 times a day).

	real	1	2[1]	3	SE
Oestrus duration (h)	46.9	46.9	46.9	46.9	
SD oestrus duration	12.4	15.8	13.4	12.8	
Freq. in optimal IO (%)	n.r.[2]	71.5	81.1	83.5	
Farrowing rate (%)	n.r.	91.4	93.3	93.7	0.2
Litter size	n.r.	12.8	12.8	12.8	0.03
Total piglet output	n.r.	11.7	12.0	12.0	0.04

[1] *2 refers to the basic situation described in the material and method section;*
[2] *n.r. means not relevant.*

Sensitivity for insemination strategy

The best reproduction results are obtained in the basic situation compared to the other scenarios (Table 7) with a farrowing rate of 93.4% and a litter size of 12.8 piglets. When insemination was applied later than in the basic situation (36 and 48 h) farrowing rate and litter size decreased to a larger extent than when insemination was applied earlier (0 and 12 h) after onset of oestrus.

Table 7. The average and SE simulated insemination to ovulation interval (IO), farrowing rate and litter size of the basic situation (24 h) and the deviation from the basic situation for the proposed scenario's (0,12, 36 and 48 h)[1] of insemination strategies applied between 0 to 48 h after detected onset of oestrus.

	0	12	24[1]	36	48	SE
IO (h)	-24.0	-12.0	-9.5	+12.0	+24.0	0.08
Farrowing rate (%)	-19.8	-4.7	93.4	-10.1	-40.4	0.3
Litter size	-0.9	-0.2	12.8	-0.2	-0.7	0.03

[1]*refers to the basic situation described in the material and method section.*

VALIDATION

Information about prenatal loss in relation to the timing of insemination relative to ovulation is scarce in literature. In most studies double or triple inseminations are applied to achieve best attainable fertilisation. In our study it is supposed that those insemination strategies are comparable with insemination applied between 0 and 24 h before ovulation. In PIGSIS, the conception rate (at least one fertilised oocyte) was 98.2% and fertilisation rate of the sows with conception was on average 94.4% when insemination was applied between 0 and 24 h before ovulation. In a study with gilts of Lambert et al. (1991), conception rate at day 3 of pregnancy was 100% and the fertilisation rate was 94.7%, which is not different from PIGSIS.

In most studies, mortality is calculated as the number of conceptuses (embryos or foetuses) divided by the number of ovulated oocytes of the pregnant gilts or sows. The embryonic, foetal and total mortality of the pregnant sows in PIGSIS was 34.9%, 3.0% and 37.9%, respectively. Variation in embryonic and foetal mortality is very high. Van der Lende and Schoenmaker (1990) reviewed embryonic mortality as reported in 78 publications of western pig breeds. In these studies with spontaneously ovulating sows (15 publications) the average number of oocytes was 16.4 ranging from 10.7 to 23.6 and the average embryonic mortality was 26.5% ranging from 19.6% to 36.8% between experiments. Although the number of ovulated oocytes is high in PIGSIS compared to their study, embryonic mortality was within the range of those sources. Van der Lende and Schoenmaker (1990) referred to 4 publications on sows with induced ovulation. The number of ovulated oocytes of those sows were comparable to the number of oocytes in PIGSIS and was on average

22.0 (range 12.2 to 25.1) and the average embryonic mortality was 30.8% ranging from 18.0% to 39.0%. Pere et al. (1997) studied embryonic and foetal mortality in the same animal by using laparotomy at day 35 of pregnancy in gilts that ovulated on average 17.4 oocytes. In the control group the embryonic mortality before 35 days was 32%, foetal mortality (between day 35 and 112) was 15% and the total mortality was 46%. Foetal mortality in PIGSIS is rather low compared to the study of Pere et al. (1997). Lambert et al. (1991) found a similar low foetal mortality (3.2%) in gilts, in accordance with results of PIGSIS. The embryonic mortality in the study of Lambert et al. (1991) of the pregnant gilts was 23.5% which, in contradiction to PIGSIS, was established by day 10 of pregnancy.

Farrowing rate in PIGSIS was 94.9% of the sows that were inseminated between 0 and 24 h before ovulation. This was already established at day 15 of pregnancy. In the study of Lambert et al. (1991), pregnancy rate at day 30 of pregnancy was 80% which was lower than in PIGSIS. In their study, pregnancy rate

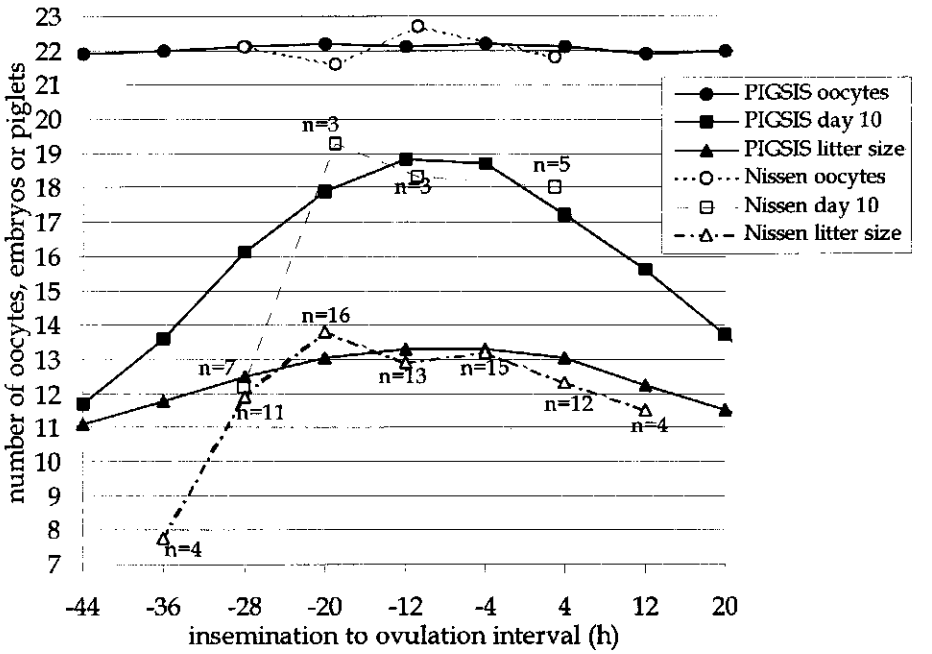

Figure 6. Number of oocytes, embryos at day 10 of pregnancy and number of total born piglets in relation to the insemination to ovulation interval as studied by Nissen et al. (1997) and the simulated results of PIGSIS.
Where n are the number of sows from the study of Nissen et al. (1997).

did not change from day 30 until end pregnancy which was also the situation in PIGSIS.

Nissen et al. (1997) studied the effects of the insemination to ovulation interval on reproduction results. They used 91 multiparous sows to study farrowing rate, 75 sows for litter size and 18 sows to study number and development of the embryos at day 10 of pregnancy. The number of ovulated oocytes was similar for both studies (Nissen et al. (1997) vs. PIGSIS) and independent of IO (Figure 6). The average number of day 10 embryos and the litter size (total number of born piglets) are at a similar level for both studies when insemination was applied between 24 h and 0 h before ovulation. When insemination is applied more than 24 h before ovulation the number of embryos at day 10 and the litter size seems to decrease more rapidly in Nissen et al. (1997) than in PIGSIS (Figure 6). In PIGSIS, the simulated farrowing rate is 95.8% when insemination was applied between 24 h and 0 h before ovulation which was 88.5% in Nissen et al. (1997) (n=52) (Figure 7). Farrowing rate decreases

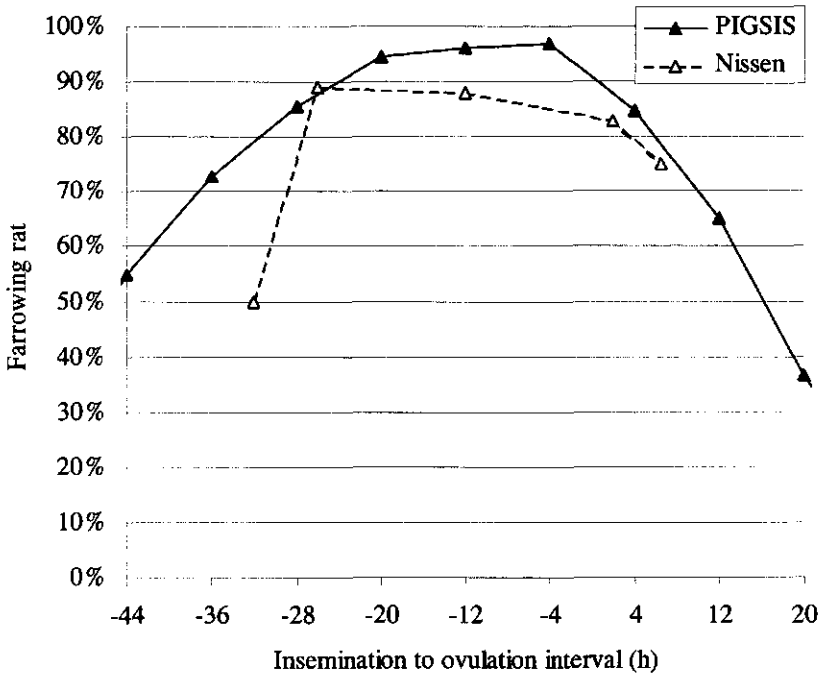

Figure 7. Farrowing rate in relation to the insemination to ovulation interval as studied by Nissen et al. (1997) and the simulated results of PIGSIS.

when insemination is applied more than 24 h before ovulation or after ovulation. A similar decrease in farrowing rate was seen when insemination was applied between 0 and 8 h after ovulation, -8% in PIGSIS and -10% in Nissen et al. (1997).

DISCUSSION

Verification and validation

From the verification it can be concluded that the used scenarios gave realistic levels for farrowing rate and litter size, within ranges as found at commercial farms. In the Netherlands, twenty percent of the commercial farms with the lowest farrowing rate on average had a farrowing rate of 74% and the twenty percent best farms on average had 90% (Siva, 1999). The twenty percent commercial farms with the lowest litter size (live and death piglets born) on average had 11.0 piglets and the twenty percent best farms had on average 12.7 total born piglets (Siva , 1999). The basic situation of PIGSIS simulated a litter size of 12.9 piglets when insemination was applied between 0 and 24 h before ovulation, which is similar to the 20% best farms in the Netherlands. This interval of 0 to 24 h is chosen because it can be considered as a good timing of insemination and reproduction results are expected to be 'optimal'. The level of farrowing rate and litter size in PIGSIS are relatively high compared to the levels found at commercial farms. This high level in PIGSIS may be caused by the fact that results of PIGSIS are obtained with only the sows that are inseminated in the optimal time before ovulation (insemination between 0 and 24 h before ovulation. At commercial farms and in literature, in which ovulation is not controlled, it can be expected that there will be more variation in the IO and that there are sows that are inseminated outside the range of the optimal IO. Another reason for the high levels of farrowing rate and litter size of PIGSIS can be that at commercial farms other management strategies than insemination strategies (nutrition, stress) or state of health (Heinonen et al., 1998), could cause a decrease in farrowing rate or litter size because of extra mortality due to: abortions or increased embryonic or foetal mortality. This is not taken into account in PIGSIS. It is also possible that the parameters like e.g. number of ovulated oocytes or uterine capacity have different levels related to management factors (e.g. feeding) or genetic background (Johnson et al., 1999).

Embryonic and foetal mortality in literature is most often studied in gilts (reviewed by van der Lende and Schoenmaker, 1990). Embryonic and foetal mortality might have different levels for sows and gilts and can have different biological backgrounds because gilts ovulate on average less oocytes than sows. Many studies on prenatal loss are done more than 10 years ago and the genetic potential of pigs in e.g. number of ovulated oocytes and uterine capacity is changed by, for example, selection on litter size. Literature in the study of Van der Lende and Schoenmaker (1990) are publications from 1954 to 1986 and the information used in PIGSIS are mainly based on sows and studies from 1994 to 1997. Validation with publications from different decades or from publications with animals from different ages (gilts vs. sows) has to be done with caution.

Effect of IO on farrowing rate and litter size

The number of embryos at day 1, 5 and 10 of pregnancy are clearly related to IO. After day 10 of pregnancy this effect of IO on the number of conceptuses decreased (Figure 5) which can be seen as the less parabolic line when conceptuses are older than 10 days. This decrease of the effect of IO is due to (1) the ending of pregnancy when not enough embryos are present in the uterus around day 14 of pregnancy (no maternal recognition); (2) a higher rate of embryonic and foetal mortality in the larger litters which results in less variation and more similarity in the litters between sows. Steverink et al. (1999b) related the results of litter size and farrowing rate of 55 commercial farms to the interval of insemination to the expected ovulation. In their study, ovulation was not obtained but the moment of ovulation was estimated at 68% of the duration of oestrus. In those farms, litter size decreased on average with 0.5 piglet when sows were inseminated between 12 h before ovulation to 12 h after ovulation (Steverink et al.,1999b). This was in agreement with PIGSIS were the decrease was 0.6 piglets. In both studies IO had a small effect on litter size when insemination was applied between 24 h before ovulation to 12 h after ovulation. The litter size in the study of Nissen et al. (1997) also showed a less pronounced relation with IO compared to the number of embryos at day 10 of pregnancy. Nissen et al. (1997) concluded that the optimal moment of insemination was between 28 h before ovulation and 4 h after ovulation, based on litter size and farrowing rate. Soede et al. (1995a) defined a smaller interval were good results could be obtained which was were insemination was between 24 h to 0 h before ovulation

which was based on reproduction result at day 5 of pregnancy. The decrease of litter size, when insemination was performed between 24 and 36 h before ovulation, seemed to be more affected by IO in Nissen et al. (1997) (4.1 piglets) than in PIGSIS (0.5 piglets). Their low number of piglets might be explained by coincidence because of the low number of animals (n=4) inseminated between 24 h and 36 h before ovulation. Moreover a relative early insemination (more 24 h before ovulation) results in a high number of sows with partial fertilisation (Soede et al., 1995a; Steverink et al., 1999a). Kemp and Soede (1997) concluded that litters with partial fertilisation have slightly retarded embryo development and increased variation in embryo development. Variation of embryo development is supposed to cause increased embryo mortality (Pope et al., 1990). In PIGSIS this aspect is not considered, which could result in an underestimation of the effects of IO on litter size.

The effect of insemination to ovulation interval (IO) on farrowing rate was more pronounced than on litter size in PIGSIS. The farrowing rate in PIGSIS was already established at day 15 of pregnancy. After day 15, mortality affected only large litters which did not result in a complete termination of pregnancy in sows. Termination of pregnancy due to, amongst others, abortions was not taken into account in PIGSIS, because information was scarce and because it was supposed to be independent of insemination strategies. Steverink et al. (1999b) related the results of farrowing rate of 55 commercial farms to the interval of insemination to the expected ovulation. In their study, ovulation was not obtained but the moment of ovulation was estimated at 68% of the duration of oestrus. In those farms, farrowing rate was more related to IO than litter size of the sows that received a single insemination, which was in accordance with results of PIGSIS.

From the sensitivity analyses one could conclude that in the basic situation, the 3 used biological parameters: ovulated oocytes, embryonic and foetal uterine capacity, are in balance. An increase in the level of one of these parameters did not increase litter size and farrowing rate to a large extent. For example, the effect of an increase in the number of ovulated oocytes was weakened because of the limitation of the large litters due to uterine capacity. This was also seen in a selection experiment on ovulation rate of Johnson et al. (1999). The increase in ovulation rate of 11 generations was 7.4 oocytes which resulted in an increase in litter size of 2.3 piglets. The increased number of oocytes with 7.4 oocytes in the sensitivity analyses

(Table 2) resulted in an increased of litter size of 1.5 piglet. It can be hypothesised that the parameters that affect litter size used in PIGSIS are in equilibrium. Selection on number of oocytes, without increasing uterine capacity, will result only in a small positive effect. When an increase of litter size and farrowing rate is desired, parameters like number of ovulated oocytes, embryonic and foetal uterine capacity need to be increased at the same time.

The differences in oestrus duration or insemination strategy affect the reproduction results by affecting the insemination to ovulation interval of the herd (average and variation). It will depend on the level of the specific farm parameters (oestrus duration, relation WOI and oestrus duration, etc.) to what extent insemination strategies will influence reproduction results. Flowers et al. (1998) applied a control insemination strategy (every 24 h during oestrus) and an adapted insemination strategy at two farms. On only one of those farm differences between treatments were observed. The differences between the two farms can be explained due to a different relation of oestrus duration and weaning to oestrus interval for those two farms. Changing the oestrus detection frequency from 3 to 1 times daily had no dramatic changes in reproduction results and the percentage not detected sows (silent oestrus) on a farm with an average oestrus duration of 48 h. However, on farms with a lower average oestrus duration an increase of sows with silent oestrus will be expected with a decrease in oestrus detection frequency.

In conclusion, a model like PIGSIS gives insight in the reproduction process due to combining information of different physiological processes. PIGSIS is a basis from which a decision support program for managing insemination strategies specific for each farm can be distilled.

IMPLICATIONS

The PIG Simulation model for Insemination Strategies (PIGSIS) can be a helpful tool to increase insight in the physiological processes of reproduction, for defining an efficient insemination strategy and for evaluating an insemination strategy as used on a farm. This model enables to define an insemination strategy adapted to the circumstances on a farm. Farms have to record the average oestrus duration, relation between oestrus duration and weaning to oestrus interval and the

frequency of weaning to oestrus interval which characterise the individual farm. The proposed simulation model can be extended into a practical implementation at farm level.

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5 General discussion

General discussion

INTRODUCTION

Reproduction efficiency in pigs shows a large variation between farms (Clark and Leman, 1987; Stein et al., 1990; Dewey et al., 1995). In the Netherlands, twenty percent of the commercial farms with the lowest farrowing rate average had an average farrowing rate of 74% and the twenty percent best farms on average had 90% (Siva, 1999). This variation was also seen in litter size, twenty percent of the commercial farms with the lowest litter size (live and dead born piglets) had an average of 11.0 piglets and the twenty percent best farms had an average of 12.7 total born piglets (Siva, 1999). The origin of the variation between farms, with respect to these reproduction results, is very complex. Factors like health status, husbandry system, management and breed can influence reproduction results. One of the management factors is timing of insemination, which can influence reproduction results by affecting fertilisation.

The research described in this thesis studied the possibility of developing a method to optimise insemination strategies for an individual farm. There are two important issues to consider in the development of an insemination strategy: (1) the effect of the moment of insemination in relation to ovulation interval (IO) on fertilisation results, and (2) possibilities to predict the moment of ovulation in order to adapt the moment of insemination to it. These two parts are the basis of the simulation model, which enables studying and defining optimal insemination strategies.

In this chapter, the history of the timing of insemination during the last decades is described as an introduction. The ideas about timing of insemination have been changed through the years. After this introduction, firstly, the sensitivity of the relation between insemination to ovulation interval and fertilisation will be discussed. Secondly, the possibilities of predicting the moment of ovulation from oestrus duration will be discussed. Thirdly, the development of the pig simulation model for insemination strategies (PIGSIS) will be discussed.

History of the timing of insemination

Timing of mating or timing of artificial insemination (AI) has always been an important issue. One of the earliest studies on AI was done with 100 to 150 ml of diluted semen (dilution 1:3) with the result that about 70% of the sows became pregnant (Rodin and Liptatov, 1935). The authors suggested that the best results would be obtained when pigs are inseminated on the second day of oestrus.

In the fifties, fertilisation results were related to the interval of insemination relative to ovulation. It was thought that ovulation occurred at about the middle of oestrus, and generally between 24 and 36 h after the onset of oestrus (Polge , 1956). The best time for insemination was said to be between 6 and 12 h before the moment of ovulation. In those days, the advice for the moment of AI was that it should take place on the second day of oestrus.

In 1970, Dziuk studied the optimal moment for insemination by using heterospermic inseminations (two inseminations of semen from two different boars). He reasoned that the semen inseminated closest to the optimum time would account for a greater proportion of offspring as compared to semen used at a less favourable time. Gilts, in which ovulation was controlled by human chorionic gonadotophin (hCG), were naturally mated twice with an interval of 6 h. In four groups of gilts that had the first mating at 30 to 24, 20 to 16, 14 to 12 and 10 to 6 h before expected ovulation, the percentage of offspring of the first boar was 33%, 30%, 78% and 71%, respectively. It was concluded that there was an optimum time point for insemination and it appeared to be about 12 h before ovulation. In that study it was striking that the latter two intervals (14 to 12 and 10 to 6 h) had better pregnancy rates: 100% and 91%, than the earlier two intervals (30 to 24 and 20 to 16 h) 67% and 69%, respectively. Furthermore, insemination closest to ovulation (10 to 6 h) resulted in the highest litter size (9.3 piglets, which was 1.1 to 2.0 piglets higher than the other 3 groups). From this it could be concluded that a first insemination from 10 to 6 h before ovulation, with a second insemination 6 h thereafter, gave the best reproduction results irrespective of the boar that sired the offspring.

Until the nineties, studies on the assessment of the optimal moment of insemination were based on litter size and farrowing rate. In the early nineties ultrasonography became a usable tool to determine the moment of ovulation transrectally (Soede et al., 1992) or transcutanously (Weitze et al., 1994; Waberski et al., 1994ab). Ultrasonography gave the possibility to study fertilisation results in pigs

with spontaneous (non-controlled) ovulation, without affecting sows by surgery and anaesthesia which might influence the fertilisation process. The results of insemination were also studied sooner after ovulation (at 5 days) instead of at farrowing, which enables to measure the direct effects of fertilisation. Using ultrasonography, Soede et al. (1995a) concluded that the best fertilisation results were obtained when insemination was performed between 24 and 0 h before ovulation based on number of embryos at day 5 of pregnancy. In a similar study with gilts in which ultrasonography was used and fertilisation results were obtained at 2 to 4 days after ovulation, an optimal insemination time was found between 12 and 0 h before ovulation (Waberski et al., 1994a). In a later study with gilts no differences in fertilisation results were obtained when insemination was applied between 12 and 24 h before ovulation compared to fertilisation results when insemination was applied between 0 and 12 h (Waberski et al., 1994b). Nissen et al. (1997) used transrectal ultrasonography and found the highest reproduction results when insemination was applied between 28 h before and 4 h after ovulation based on embryos at day 10 of pregnancy, farrowing rate and litter size.

At this moment it is clear that a large part of the variation in fertilisation results is related to the interval between insemination and ovulation. Desirable fertilisation results can be obtained when insemination takes place between 0 and 24 h before ovulation, under present circumstances.

FERTILISATION IN RELATION TO INSEMINATION AND OVULATION

From the above mentioned it is clear that the moment of insemination in relation to ovulation has a great impact on the fertilisation results. Variation can be studied and quantified by using mathematical model, which is developed in Chapter 2.3. Moreover, it is of interest what the physiological mechanism is explaining this relation and to what extent this relation can be influenced by factors like e.g. quantity of semen, quality of semen and sow effects.

Physiological background

At insemination or mating in pigs, billions of sperm cells are deposited in the uterus near the cervix. From this site, spermatozoa start to migrate towards the site of

sperm storage which is in the first 2 cm of the caudal region of the isthmus in the oviduct (Hunter, 1981). Longitudinal contractions of the uterus are responsible for transport of the spermatozoa (Zerobin, 1968; Bower, 1974). Relative to the inseminated number of sperm cells, only a small number reaches the isthmic sperm reservoir (Hunter, 1981). Before the sperm cells reach the oviduct they have to pass a major barrier: the uterotubal junction (UTJ) (Smith et al., 1987). Dead (Viring, 1980) or capacitated spermatozoa (Shalgi et al., 1992) pass the UTJ not as good as normal spermatozoa. When sperm cells reach the reservoir, they can be stored for a certain period which delays the reduction of the motility, viability and fertilisation capacity (Overstreet et al., 1980; Suarez et al., 1991). This makes the reservoir a temporal shelter to bridge the time until fertilisation of oocytes. Sperm cells can be stored in the reservoir for up to 40 h (Hunter, 1981; Pollard et al., 1991; Raycoudhurry and Suarez, 1991). However, a general decrease in number of fertile sperm cells is seen during storage in the sperm reservoir. Spermatozoa that do not reach the sperm reservoir in time are killed by the hostile uterine environment. In the hamster uterus, the motility of spermatozoa decreases from 60% immediately before insemination to 10% at 1 h after insemination (Smith et al., 1988). The spermatozoa that do not reach the sperm reservoir, are removed by backflow (Viring and Einarsson, 1981; Chapter 2.2) or local phagocytosis which is seen within 2 h after insemination (Pursel et al., 1978).

From the described above it can be concluded that the relation between IO and fertilisation is based on the ageing processes of sperm cells and oocytes. When insemination takes place before ovulation, the sperm cells will age. The ageing process of sperm cells causes a decrease of the number of sperm cells in the sperm reservoir. When insemination takes place after ovulation the oocytes will age until fertilisation. Both ageing processes cause a decrease in the chances of fertilisation. This means that the moment of releasing sperm cells in the female reproduction tract (insemination) in relation to the moment of releasing the oocytes (ovulation) determines the period in which fertilisation is possible.

Factors affecting the relation between IO and fertilisation

As mentioned before, Soede et al. (1995a) concluded that the best fertilisation results were obtained when insemination was performed between 24 and 0 h before ovulation. In their study sows were inseminated with a normal commercial sperm dosage of good quality containing 3×10^9 sperm cells. This means that given these

circumstances ageing of sperm cells becomes visible when sperm cells are stored for longer than 24 h in the sperm reservoir. The effect of ageing of sperm cells on fertilisation might be changed by different circumstances. Chapter 2.1 and 2.2 explores factors that can be seen as changed circumstances compared to the study of Soede et al (1995a).

Quantity of semen: In Chapter 2.1 three different sperm dosages are compared and differences of fertilisation were obtained 5 days after ovulation. Insemination at 12 to 24 h before ovulation, with a threefold reduced sperm dosage (1×10^9 sperm cells) did not result in a significant reduction in fertilisation results in sows. Insemination at 24 to 36 h before ovulation with a twofold increased sperm dosage (6×10^9 sperm cells) did not result in an increase in fertilisation results. However, small consistent differences were seen, both the median percentage of normal embryos and the median accessory sperm count increased with an increase in insemination dosage in all the insemination to ovulation classes 0-12 h, 12-24 h, 24-36 h and 36-48 h. These results indicate that fertilisation results are not very sensitive to variation in the number of inseminated sperm cells in the range of 1×10^9 to 6×10^9 sperm cells. This will mean that the number of fertile sperm cells in the sperm reservoir is not much affected in the range of 1×10^9 to 6×10^9 sperm cells.

In Chapter 2.2, the influence of backflow of semen on fertilisation rate was studied. The hypothesis was that backflow of semen in the first half hour after insemination would negatively influence the number of sperm cells that could reach the sperm reservoir and consequently would negatively influence fertilisation. The volume of backflow up to 2.5 h after insemination was 70% of the inseminated volume, which was relatively high compared to the number of sperm cells in backflow that was 25% of the inseminated sperm cells. When sows had more than 5% backflow of the inseminated volume, then the fertilisation results were negatively affected in the sows inseminated with 1×10^9 sperm cells. This was not seen with an insemination dosage of 3×10^9 or 6×10^9 sperm cells. However, no effect of backflow after insemination on fertilisation results was found. It could be concluded that suboptimal circumstances like a combination of low dosage and loss of spermatozoa due to backflow during insemination, will lead to sub-optimal fertilisation results. This will mean that the combination of two suboptimal factors will lead to a less good filling of the sperm reservoir and therefore cause less good fertilisation results.

Quality of semen: The quality of semen (e.g. fertilisation capacity, morphology), is different among boars (Harkema et al., 1997). The quality of semen is also affected by the longterm storage and cryopreservation (cell damage, motility). Cryopreservation and long term storage cause cell damage which results in a higher number of dead cells and a higher elimination rate of sperm cells in the female reproductive tract (Pursel et al., 1978; Saacke, 1982).

Waberski et al. (1994a) studied the effect of timing of insemination in relation to ovulation of frozen stored semen compared to fresh (stored for less than 48 h) semen in gilts on fertilisation results between 2 and 4 days after ovulation. The optimal insemination time with cryopreserved semen was 0 and 4 h before ovulation in which good (more than 80%) fertilisation results could be obtained. This period was shorter compared to fresh semen which had an optimal insemination time of 0 to 12 h before ovulation.

Longterm storage of semen decreases the fertilisation capacity of the sperm cells. The use of longterm stored semen (48 to 87 h) decreased the interval from insemination to ovulation in which good fertilisation results (more than 80%) could be obtained from 0 to 24 h to 0 to 12 h compared to fresh semen (Waberski et al., 1994b). A further increase of storage time of semen (87 to 118 h) resulted in lower fertilisation rate (73.0%) even in the insemination to ovulation interval of 0 to 12 h.

Summarising, it can be concluded that the quality of semen affects fertilisation result clearly. The IO in which good fertilisation results can be obtained is shortened. Cryopreservation or storing of semen for more then 48 h result in cell damage or dead sperm cells which result in less fertile sperm cells at the side of fertilisation compared to good quality semen.

Sow effects (parity, breed): Beside semen characteristics that influences the relation between IO and fertilisation, individual sow factors can also play a role. These factors can be e.g. the parity or the genetic background of a sow.

In the experiment described in Chapter 2.1 sows of parity 1 to 8 were used. In those sows, a small but significant effect of parity was found on the relation between IO and fertilisation results (unpublished results, Steverink DWB). Sows of parity 3 and older on average have a better fertilisation rate. When insemination was applied between 12 and 24 h, before ovulation the average fertilisation rate for parity 1, 2 and older sows was 85.9%, 84.2% and 90.5%, respectively. When insemination was

applied between 24 and 36 h before ovulation, the average fertilisation rate for parity 1, 2 and older sows was 77.3%, 67.4% and 84.7%, respectively. In contradiction to this, Soede et al., (1995a) found no difference in fertilisation rate between parity 2 and older sows (5 parity 1 sows, 55 parity 2 sows and 83 parity 3 and older sows). In a study in which gilts were used, the optimal period of insemination relative to ovulation was between 0 to 12 h (Waberski et al., 1994a). This was a shorter IO than in studies where sows were used (Chapter 2.1; Soede et al., 1995ab). However, in another study with gilts no difference in fertilisation rate were found when insemination was applied between 0 and 12 h before ovulation compared to insemination between 12 and 24 h before ovulation (Waberski et al. (1994b). Difference in fertilisation rate due to parity is conflicting in literature. It seems however that the younger sows and gilts have a small disadvantage in fertilisation results. Differences due to age might be related to a suboptimal sperm transport in the uterus (e.g. worse uterine contractions). However, no such effects have been reported.

The variation in fertilisation rate as described in Chapter 2.1 partly had a genetic basis. The fertilisation rate was positively correlated (R^2=26%, $P<0.01$) to the breeding value for litter size of the sows. This means that the fertilisation rate at day 5 of gestation is related to the number of piglets the sow potentially can produce. A prolongation of the optimal insemination to ovulation interval is seen in sows with a high breeding value for litter size. A similar effect was seen when the fertilisation results of two breeds (dam and sire line) were compared (Kemp and Soede, et al., 1997). When insemination was performed between 0 and 12 h after ovulation the dam-line showed still good fertilisation (98.7%; n=11) whereas the sire-line showed a marked decline in fertilisation (68.5%; n=33)($P<0.05$). It could be concluded that selection for reproduction parameters has a positive effect on the fertilisation and that, consequently, the insemination to ovulation interval is longer for dam-lines than for sire-lines in which good fertilisation results will be obtained.

In conclusion, it appeared that age and genetic background of the sow affect the fertilisation results as measured at day 5 of gestation. Both, sows that are selected for reproduction performance and sows of older age have a larger IO in which good fertilisation results can be obtained in comparison to younger sows and animals that are selected for production traits.

Mathematical model of conception and fertilisation

From the reported studies it became clear that there is a high variation in fertilisation rate among sows (Chapter 2.1). Fertilisation is not an all or non phenomenon which it was thought to be for a long time (Hunter, 1994), but sows also can have partial fertilisation. Fertilisation has to be described more accurately to estimate the impact of IO on the number of fertilised oocytes per sow. A mathematical model of conception and fertilisation was developed based on data obtained under 'normal' conditions in the Netherlands (Chapter 2.3). These conditions consist of using multiparous sows and insemination with a commercial sperm dose of 3×10^9 sperm cells which is stored for less than 48 h and with sperm cells of proven quality. Figure 1 shows the estimated relation of conception and fertilisation under these 'normal' conditions. At the moment, there are not enough data available to estimate the

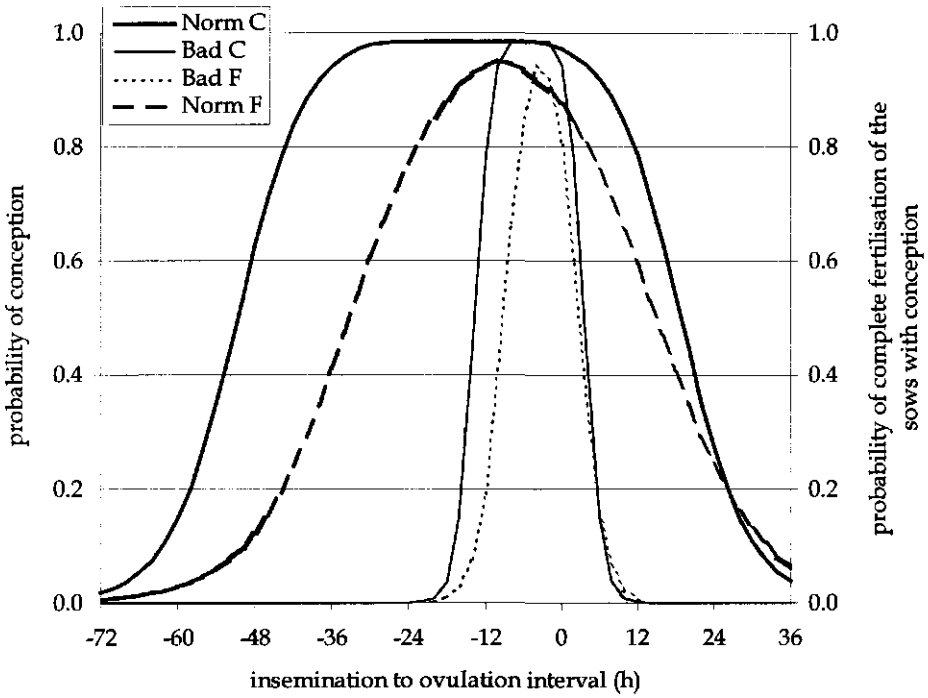

Figure 1. The estimated probability of conception (C; ---) (at least one embryo) and complete and almost complete fertilisation (F; - - -) of the sows with conception in relation to the interval between insemination and ovulation for normal (Norm) insemination conditions and suggested probability conception and fertilisation for bad (Bad) insemination conditions.

parameters for the mathematical model on fertilisation under less optimal conditions (e.g. insemination with frozen stored sperm cells). In Figure 1, an example is given of how negative factors (bad conditions) might affect the conception and fertilisation results. As was suggested in Chapter 2.3 the insemination to ovulation interval in which good conception and fertilisation results can be obtained is shorter under these bad conditions but the highest level is similar in both situations. If more information about conception and fertilisation becomes available under specific circumstances, the mathematical model could be adapted to it.

From Chapter 2.2 it was concluded that a low dosage of semen (1×10^9 sperm cells) hardly affected fertilisation results. However, a low dosage of semen in combination with excessive backflow during insemination had a negative influence on fertilisation. This in contrast to a normal or high dosage of semen in combination with high volume of backflow which did not affect fertilisation. From this it might be concluded that negative effects of one factor can be partly compensated by changing one of the other factors. Moreover, it is of interest whether negative factors, like frozen stored semen, could partly be compensated by using positive factors like older sows or dam-line sows.

The mathematical model represents the results of fertilisation using a single insemination. In the Netherlands this is not common practice where on average 1.5 inseminations per oestrus period are used (Siva, 1999). The effect of a double insemination in relation to the optimal insemination to ovulation interval has hardly been studied. Soede et al. (1995b) applied a second insemination between 0 and 5 h after ovulation. They found good fertilisation results, irrespective of the timing of first insemination relative to ovulation. In this study, it seems that the later insemination compensated a first insemination, when the first one had been performed too early (insemination 32 to 24 h before ovulation) without an adverse affect of the second insemination. From this it could be suggested that, when a double insemination is applied, the one with the highest probability on good fertilisation results is the one responsible for the fertilisation results.

In conclusion, fertilisation is a complex process resulting in no, partial or complete fertilisation. The mathematical model in Chapter 2.3 describes this process accurately for 'normal' insemination conditions. Although the variation in fertilisation in relation to insemination and ovulation is described, the origin of this variation is still not understood very well. Deviations from the proposed

mathematical model are expected when circumstances are substantially different from these 'normal' conditions.

POSSIBILITIES OF PREDICTING OVULATION

General

From the foregoing it can be concluded that the moment of ovulation is a crucial moment for timing of insemination. This is because fertilisation results depend on the insemination to ovulation interval. Therefore, prediction of ovulation is a prerequisite for optimising insemination strategies.

At commercial farms the timing of insemination is based on the onset of oestrus. However, ovulation takes place at very variable moments after the onset of oestrus (detected as standing response for the boar). The ovulation moment varied between 10 and 85 h after the onset of oestrus (Soede and Kemp, 1997). Because of this variation, onset of oestrus is not an accurate predictor for the moment of ovulation and therefore not a good parameter for timing of insemination.

Many possible ovulation predictors have been studied. Stokhof et al. (1996) studied vaginal mucus conductivity. The vaginal mucus conductivity increased during oestrus but the variation between sows was very high and there was no relation with the observed ovulation moment as determined with ultrasonography. Soede et al. (1997) studied vaginal temperature from 4 days before ovulation to 2 days after ovulation in 10 sows. In these sows a clear day/night rhythm in vaginal temperature was found but a relation with the ovulation moment could not be demonstrated. Soede et al. (1994) studied concentrations of oestradiol, LH and progesterone in relation with the ovulation moment. The preovulatory LH surge is a good ovulation predictor but there is not a simple practical test available to determine the preovulatory LH surge at this moment. Ultrasonography is a tool to detect the ovulation moment retrospectively. A prediction of ovulation based on the follicle diameter is not possible because of high variation. The size of follicles at the moment of ovulation varied between 5 and 10 mm (unpublished data, Steverink DWB). Nissen (1995) found that the follicles reached a maximum diameter of 7-10 mm, at which size they remained for about 24 h until ovulation. None of these

parameters can predict ovulation accurate enough to be used for timing of insemination.

Oestrus duration

Oestrus duration is a retrospective estimator of ovulation that might be used in a prospective way. Table 1 shows oestrus and ovulation data from 6 different studies in which ovulation was detected by ultrasonography (Weitze et al., 1994; Nissen et al., 1995; Mburu et al., 1995; Soede et al., 1995a; Soede et al., 1995b; Chapter 2.1). In these studies the mean oestrus duration ranged between 56 and 60 h and the weaning to oestrus interval between 86 and 124 h. The mean relative ovulation time during oestrus varied between 67% and 72%, and ranged between 35% and 163%. Only a few sows had a relative ovulation of more than 100% (ovulation after oestrus) and these were sows with an oestrus duration which was shorter than 32 h (Soede et al., 1995a; Chapter 2.1). From these experimental studies it can be concluded that oestrus duration can be used as an estimator of the moment of ovulation but unfortunately this estimator is a retrospective one.

Table 1. Relative ovulation (%) during oestrus and the oestrus duration (h).

Relative ovulation (%)			Oestrus duration	
Mean±sd	Range	N	Mean±sd	Reference
71±n.d.[a]	35-100	483	60±15	Weitze et al., 1994
71±14	38-118	91	60±14	Nissen et al., 1995
68±8	54-78	20	56±8	Mburu et al., 1995
72±15	39-133	144	50±13	Soede et al., 1995a[b]
67±8	42-94	91	60±11	Soede et al., 1995b
68±10	46-163	115	59±12	Chapter 2.1[c]

[a] *n.d. not determined*
[b] *Sows with a WOI of more than 8 days were excluded (n=2)*
[c] *Sows with a WOI of more than 7 days were excluded (n=19)*

Oestrus duration at farm level

From Chapter 3.1 it can be concluded that the average oestrus duration on farms was different between farms but consistent from month to month within a farm with a repeatability of 86%. Therefore, recording of the duration of oestrus at farms for approximately a month can give a good impression of the duration of oestrus for the coming period on that farm.

Oestrus duration is negatively related to the weaning to oestrus interval (Chapter 2.1; Weitze et al., 1994; Kemp and Soede, 1996). In Chapter 3.1 it was also shown that the relation between oestrus duration and weaning to oestrus interval differs among farms. Moreover, a difference in the average weaning to oestrus interval among farms was found. When the insemination strategy will be based on oestrus duration at a farm, these specific farm characteristics like average oestrus duration and the relation of weaning to oestrus interval and oestrus duration need to be taken into account.

Oestrus duration on sow level

An insemination strategy specific for each sow would be the ultimate goal for optimising timing of insemination. This will be possible when the moment of ovulation is predictable for an individual sow. The question is if oestrus duration is repeatable for sows. The heritability was 0.16 for oestrus duration and .29 for the ability to show standing reflex (Rydhmer et al., 1994). From this it can be concluded that a genetic component of oestrus duration is present in pigs. In a pilot study, data on oestrus duration were collected of 153 sows from 1995 to 1997 at one commercial farm (unpublished results, Steverink). Sows with more than 3 reproductive cycles were included in the analysis. From this pilot study it was concluded that the repeatability of oestrus duration was between 0.16 and 0.22 at this farm. With a repeatability of around 0.2, oestrus duration is not predictable accurate enough for sow. Oestrus duration and its predictability should be studied further. Possibly more parameters should be obtained in order to predict oestrus duration more accurate for individual sows.

DEVELOPMENT OF A MODEL FOR INSEMINATION STRATEGIES

From the above it can be concluded that there are two issues to consider when developing a method to optimise insemination strategies in pigs. At the first place fertilisation results as depending on the interval between insemination and ovulation and in the second place possibilities to predict the moment of ovulation in order to adapt the moment of insemination to it. Combining these factors and also the factors that are involved in embryonic and foetal mortality makes a model approach of

interest. A model is a good aid to investigate the impact of variation of certain input factors on the reproduction outcome. Moreover, putting down existing knowledge into a simulation model would result in a valuable tool for educational purposes.

In literature two models have been published which studied reproduction results by using underlying physiological mechanisms. One model is based on the concept that litter size is the product of ovulation rate and embryonic survival (Johnson et al., 1984). In the other model, litter size is based on the concept of ovulation rate and uterus capacity (Bennet and Leymaster, 1989). The major shortcoming of these models is caused by the fact that fertilisation can not be distinguished from mortality during pregnancy. The effect of insemination can therefore not be studied by these two models. Moreover, the impact of insemination strategies on the change in number of embryos and foetuses at several stages in pregnancy can not be detected. Therefore, a new mathematical model was introduced.

Chapter 4.1 proposed this new model and it can be concluded that the basic model reasonably simulated reproduction results. The sensitivity analysis indicated that PIGSIS is a robust model because it did not generate exceptional results when values of the parameters were changed.

Illustration of the effect of a similar insemination strategy on two different farms

To illustrate the importance of an insemination strategy for an individual farm a simulation study was done with a preliminary version of PIGSIS (Steverink et al., 1999). The objective of that study was to show the consequences for reproduction results when applying the same insemination strategy on farms with a different average oestrus duration. One simulated farm had an average oestrus duration of 36 h and the other farm had an average oestrus duration of 60 h. The applied insemination strategy consisted of a single insemination 24 h after observed onset of oestrus. In this study it was concluded that the farm with the short average oestrus duration had a lower farrowing rate than the farm with the long average oestrus duration, 79% and 86%, respectively. The litter size was not different between the two farms. The origin of the difference was the shift in insemination to ovulation interval (Figure 2). At the farm with an oestrus duration of 60 h, more sows were inseminated more than 24 h before ovulation, while at the farm with an oestrus duration of 36 h more sows were inseminated after ovulation. The percentage of

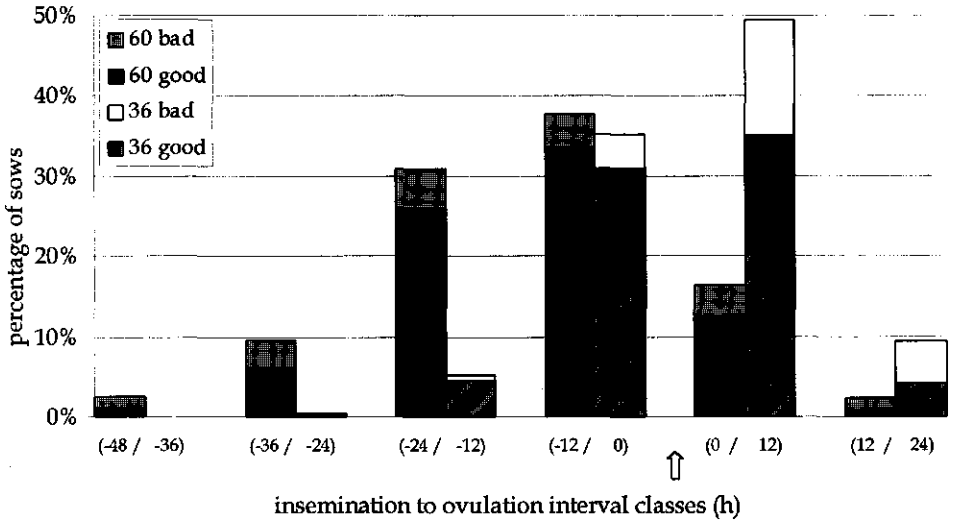

Figure 2. Percentage of sows in the farm with an average oestrus duration of 60 and 36 h with bad (0-80%) and good (80-100%) fertilisation rate at the different insemination to ovulation intervals. ⇧ = *the moment of ovulation.*

sows inseminated in the optimal IO (0 to 24 h before ovulation) was 38% at the farm with an OD of 36 h and 68% at the farm with and OD of 60 h (Figure 2).

This simulation study is an example of the possibilities of using a model to improve the insemination strategy on a farm and an example of how the model it gives insight in the processes involved in reproduction.

Illustration of achieving information with PIGSIS

A model like PIGSIS, can be used to study fictive pig populations. The impact of number of ovulated oocytes and uterine capacity can be studied. An example of four different populations of sows is simulated by PIGSIS (Table 2): a control population as described in Chapter 4.1 as the basic situation, a population with a low number of ovulated oocytes (low OO), a population with a high embryonic

Table 2. Four simulated pig populations to study the effect of insemination to ovulation interval on reproduction results.

population	oocytes	embryonic UC[a]	foetal UC
control	basic[b]	basic	basic
low OO	basic-7.4	basic	basic
high UC	basic	basic+8.8	basic+4.0
low OO+ high UC	basic-7.4	basic+8.8	basic+4.0

[a] *UC is uterine capacity; OO is number of oocytes*
[b] *basic is basic value as described in Chapter 4.1*

and foetal uterine capacity (high UC) and a population with low OO and high UC. The effect of IO on litter size is shown in Figure 3. The two populations with a low ovulation rate result in a lower litter size. Moreover, The effect of IO on litter size is less pronounced in the two populations with the lower number of oocytes compared to the basic situation. This less pronounced effect of IO on conceptuses was already seen at day 1 and became less at day 15. The embryonic mortality in the basic population was higher compared to population with low OO and high UC (Figure 4). In the latter foetal mortality was almost absent. This example shows how ovulation rate and uterine capacity affects reproduction results.

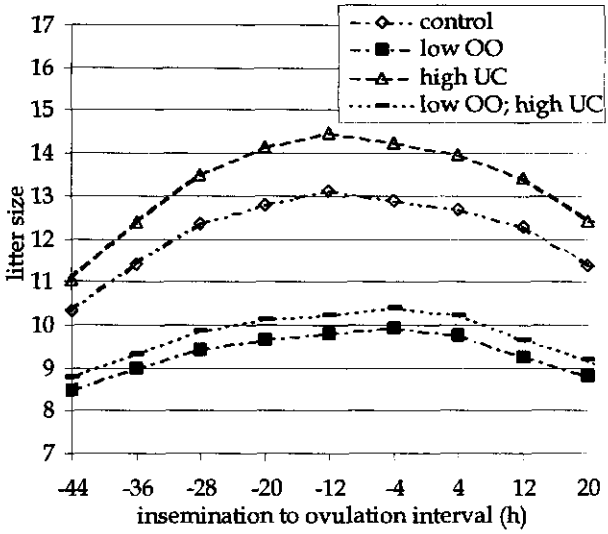

Figure 3. Simulated litter size of the control population of PIGSIS (basic), low number of ovulated oocytes (low OO), high embryonic and foetal uterine capacity (high UC) and with low OO and high UC.

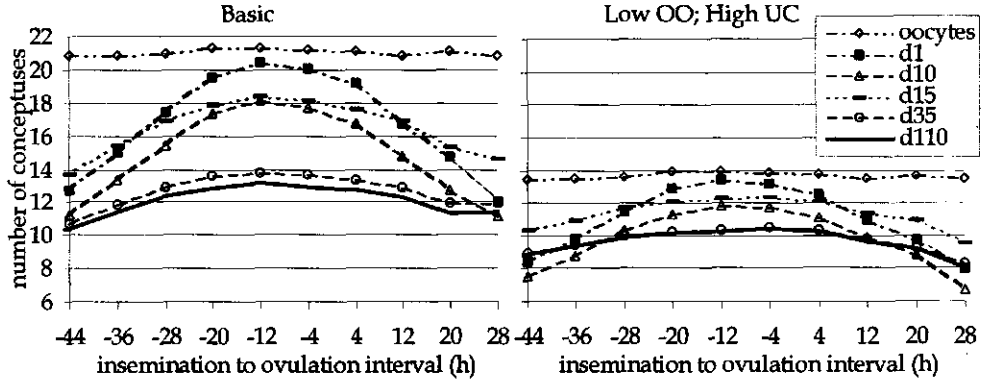

Figure 4. Number of oocytes and conspectuses for the basic population (left) and for the population with low number of ovulated oocytes (basic - 7.4 oocytes)and high embryonic and foetal uterine capacity (right) at day 1, 10, 15, 35 and 110.

Improvements of PIGSIS

Until now PIGSIS has been validated in parts with data from literature because no other data were available. A complete validation of PIGSIS as a whole is needed for a better evaluation of the model. For this further validation, data of commercial farms that apply two different insemination strategies can be used.

PIGSIS used the oestrus duration in relation to weaning to oestrus interval and the frequency of parity of the sows as input parameters. These parameters characterise the farm which makes the insemination strategy adapted to the unique situation at that farm. A further improvement of PIGSIS would be if more farm characteristics related to oestrus duration could be defined. In Chapter 3.1 it was shown that the average oestrus duration was different between farms, which is accounted for in PIGSIS. But it was also seen that the standard deviation (SD) of oestrus duration was different between farms, ranging from 8 to 18 h (unpublished results). In PIGSIS the SD of oestrus duration was 10 h and was equal for all farms. When the variation in oestrus duration at a farm is high the variation of the moment of ovulation will also be high at that farm. To inseminate all animals in a good insemination to ovulation interval will then be impossible with one insemination. An insemination strategy for farms with a high variation in oestrus duration will need more inseminations per sow than farms with a low variation in oestrus duration. To improve PIGSIS also the variation on oestrus duration can be used specific for the farm.

Instead of anticipating on the variation in oestrus duration, another approach could be to study the possibility to reduce variation in oestrus duration at a farm. Therefore, it is of interest to investigate the factors that are responsible for the difference in average oestrus duration and variation of oestrus duration between farms. Factors that are known to influence oestrus duration are e.g. stress of group-housing (Pederson et al., 1993), boar (Jongeman et al., 1996) and possibly the skills of the farmer. If underlying factors are known it might be possible to adapt the method of oestrus detection into a method in which the oestrus duration will be less variable and thus improving the possibility to identify an efficient insemination strategy at farm level.

The bottleneck of timing of insemination is the accuracy of predicting ovulation. As described before, the oestrus duration is the best retrospective ovulation estimator at this moment. However, variation was seen in the moment of

ovulation during oestrus. It will be an advantage for PIGSIS when prediction of ovulation can be done with a higher accuracy. Possibly a combination of oestrus characteristics like standing reflex in front of the boar in combination with colouring of the vulva could give a better predictor for moment of ovulation (Langendijk et al., 1999).

PIGSIS is a model which is based on sows that show their first oestrus after weaning. Gilts and rebred sows are not included in the model because prediction of oestrus duration and therefore moment of ovulation is less accurate. However when oestrus duration of these two groups is known a prediction of ovulation could be made and an insemination strategy can be defined.

An insemination model like PIGSIS, becomes of more interest when circumstances become worse. For example, when sperm dosages become more expensive or the labour costs for insemination become higher. Also when it is favourable to use a low dosage of semen due to health problems (e.g. classical swine fever) or using less boars. In these situations, applying a single insemination has an advantage over a double insemination and timing of insemination will become more crucial.

This study demonstrated the importance of taken into account the specific farm parameters when defining an insemination strategy. On farms, oestrus duration needs to be registered for a certain period of time to enable prediction of oestrus duration for the coming period. Also, the relation of oestrus duration and weaning to oestrus interval should be registered on farms. With these two parameters, a specific insemination strategy can be formulated adapted to circumstances on the farm. PIGSIS is a basis from which a software application for use at commercial farms could be distilled.

Models in scientific research are used to gain insight in the processes under study. In PIGSIS assumptions are made and not all of them could be validated because data were not available. Therefore, PIGSIS is still in its developing stage and reservation has to be taken into account by using PIGSIS, at this stage, as a decision supporting program. Nevertheless, the value of the current simulation model is that it helps to increase understanding and insight in the reproduction processes that are important for the insemination strategy. Directions for further research can be defined, leading to more information for optimising the insemination strategies at commercial farms.

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Summary

Summary

INTRODUCTION

Reproductive efficiency shows large variation between farms. The origin of the variation between farms, with respect to these reproduction results, is very complex. Factors like health status, husbandry system, management and breed can have an influence on reproduction results. One of the management factors is timing of insemination, which influences reproduction results by affecting fertilisation.

The research described in this thesis deals with the possibility of developing a method to optimise insemination strategies for individual farms. Therefore three objectives were formulated: the first objective is increasing insight in the effects of the interval between insemination and ovulation on fertilisation results. The second objective is increasing knowledge on the possibilities of predicting the moment of ovulation of sows at a farm. The final objective is developing a method which can be used for optimising insemination strategies at commercial farms.

FERTILISATION IN RELATION TO INSEMINATION AND OVULATION

In Chapter 2 the sensitivity of the relation of the insemination to ovulation interval (IO) and fertilisation results is studied. Fertilisation results are not very sensitive to variation in the number of inseminated sperm cells in the range of 1×10^9 to 6×10^9 sperm cells (Chapter 2.1). Sows with more than 4 ml backflow of semen during insemination had reduced fertilisation results when the sows were inseminated with 1×10^9 sperm cells, but this was not seen with an insemination dosage of 3×10^9 or 6×10^9 sperm cells (Chapter 2.2). Backflow of semen after insemination did not affect fertilisation results. It could be concluded that sub-optimal circumstances like a combination of a low dosage and loss of sperm cells due to backflow during insemination, lead to sub-optimal fertilisation results.

Fertilisation is a complex process, resulting in no, partial or complete fertilisation of the oocytes. The variation in conception (at least one oocyte fertilised) and fertilisation rate between sows is high, but a large part of the variation is related to the interval between insemination and ovulation. A mathematical model for conception and fertilisation is described in Chapter 2.3. The data used for estimating the parameters in the model were derived from multiparous sows that were inseminated once with a commercial sperm dose of 3×10^9 sperm cells of proven quality which was stored for less than 48 h and with sperm cells. In the model, the probability of conception is maximal (98%), when insemination is performed between 29 and 3 h before ovulation. The probability of complete fertilisation (all oocytes fertilised) is maximal when insemination was performed at 9.6 h before ovulation. At this optimal fertilisation point, the probability of partial fertilisation is 21% which increases beyond this point.

PREDICTION OF OVULATION

Fertilisation results are related to the interval between insemination and ovulation. Therefore, the moment of ovulation is a crucial moment for timing of insemination. Many potential ovulation predictors have been studied, but only oestrus duration is a reasonable estimate (retrospectively) for ovulation. Ovulation takes place at on average twothirds of oestrus. Unfortunately oestrus duration is very variable.

The average oestrus duration is different between farms ranging between 31 and 64 h (Chapter 3.1). Moreover, oestrus duration is consistent from month to month within a farm with a repeatability of 86%. Furthermore, oestrus duration is negatively related to the weaning to oestrus interval. This relation differs among farms. These specific farm parameters can be used to predict the oestrus duration and from that the ovulation can be predicted. These farm parameters (average oestrus duration and the relation of weaning to oestrus interval and oestrus duration) can be used to define a specific insemination strategy for each farm.

DEVELOPMENT OF A MODEL FOR INSEMINATION STRATEGIES

There are a variety of factors influencing the reproduction process. The complexity of this reproduction process makes a modelling and simulation approach valuable because effects of the underlying processes can be controlled. A PIG Simulation model for Insemination strategies (PIGSIS) was developed which consists of two parts: (1) the reproduction events from the number of ovulated oocytes until the number of piglets at farrowing and (2) timing of insemination relative to ovulation based on the farm parameters (weaning to oestrus interval, oestrus duration, etc.). PIGSIS simulates the reproduction results at day 1, 5, 10, 15, 35 and 110 of pregnancy. Many physiological processes are included in PIGSIS e.g. fertilisation, embryonic mortality (degeneration, maternal recognition of pregnancy, embryonic uterine capacity) and foetal mortality (foetal uterine capacity). After verification and validation it could be concluded that PIGSIS is a robust model that reasonably simulates reproduction results. Under the basic situation (average oestrus duration of 47 h and average parity of 4.2) and when insemination was applied between 0 and 24 h before ovulation PIGSIS simulates 12.9 total born piglets and a farrowing rate of 94.9%. Under these conditions the average embryonic and foetal mortality of the conceptuses was 34.9% and 3.0%, respectively. The effect of insemination to ovulation interval on fertilisation results is clear, but the effect becomes less clear as gestation proceeds resulting in a more pronounced effect on litter size than on farrowing rate.

In the General discussion the results of the studies are discussed and an illustration of the usability of PIGSIS is given. Verification and partial validation gave confidence in the model. However, a further validation is required to evaluate the model as a whole. Therefore PIGSIS is still in its developing stage and reservations has to be taken into account at this stage by using PIGSIS for defining optimal insemination strategies on farms.

Samenvatting

Samenvatting

INLEIDING

De variatie in reproductie efficiëntie is groot tussen bedrijven. De oorsprong van deze variatie is erg complex. Factoren zoals gezondheidsstatus, houderijsysteem, management en ras kunnen een invloed op de reproductieresultaten hebben. Een van deze management factoren is het moment van insemineren (inseminatiestrategie) welke via bevruchting een invloed heeft op de reproductie resultaten.

Het onderzoek in dit proefschrift beschrijft de mogelijkheid van het ontwikkelen van een methode om de inseminatiestrategie te optimaliseren die rekening houdt met de situatie op individuele bedrijven. Voor dit proefschrift zijn daarom drie doelstellingen geformuleerd: het eerste doel is om het inzicht te vergroten van de effecten van het interval tussen insemineren en ovuleren op bevruchtingsresultaten. Het tweede doel is om de kennis te vergroten over het voorspellen van het ovulatie moment van zeugen op bedrijven. Het laatste doel is om een methode te ontwikkelen welke gebruikt kan worden om de inseminatie strategie te optimaliseren op commerciële bedrijven.

BEVRUCHTING IN RELATIE TOT INSEMINATIE EN OVULATIE

In Hoofdstuk 2 is de relatie tussen het inseminatie tot ovulatie interval (IO) en bevruchtingsresultaten bestudeerd. Bevruchtingsresultaten bleken niet erg gevoelig voor de variatie in het aantal geïnsemineerde spermacellen wanneer inseminatie doses in de range van 1×10^9 tot 6×10^9 sperma cellen werden gebruikt (Hoofdstuk 2.1). Zeugen met meer dan 4 ml sperma terugvloei gedurende de inseminatie hadden slechtere bevruchtingsresultaten als ze geïnsemineerd waren met 1×10^9 spermacellen, maar dit werd niet geconstateerd bij zeugen die geïnsemineerd waren met een dosis van 3×10^9 of 6×10^9 spermacellen (Hoofdstuk 2.2). Terugvloei van sperma na het insemineren had geen effect op de bevruchtingsresultaten. Hieruit kan worden geconcludeerd dat suboptimale omstandigheden, zoals een combinatie van een lage spermadosis en verlies van sperma

door spermaterugvloei tijdens insemineren, tot suboptimale bevruchtingsresultaten kan leiden.

Het bevruchtingsproces is een complex proces en kan als resultaat de bevruchting van geen, een deel, of alle eicellen hebben. De variatie in de kans op conceptie (minstens één eicel bevrucht) en bevruchting tussen zeugen is groot. Een groot deel van de variatie in beide kenmerken hangt samen met het interval tussen inseminatie en ovulatie. Een mathematisch model voor deze kansen op conceptie en bevruchting is beschreven in Hoofdstuk 2.3. De gebruikte data voor het schatten van de parameters in het model zijn afkomstig van bevruchtingsgegevens van meerdere-worps zeugen die één keer zijn geïnsemineerd met een commerciële spermadosis met 3×10^9 sperma cellen die een bewezen goede kwaliteit hadden en die voor minder dan 48 uur bewaard werden. In het model is de kans op conceptie maximaal 98% wanneer de inseminatie tussen 29 en 3 uur voor ovulatie heeft plaatsgevonden. De kans op complete bevruchting (alle eicellen bevrucht) is maximaal wanneer de inseminatie 9,6 uur voor ovulatie heeft plaatsgevonden. Op dit optimale bevruchtingstijdstip is de kans op gedeeltelijke bevruchting 21%. Voor en na dit optimale tijdstip neemt het aandeel gedeeltelijke bevruchting toe ten koste van het aandeel complete bevruchting.

VOORSPELLING VAN OVULATIE

Uit het voorgaande blijkt dat bevruchtingsresultaten duidelijk gerelateerd zijn aan het interval tussen insemineren en ovuleren. Daarom is het ovulatiemoment een cruciaal moment voor het bepalen van een optimale inseminatiestrategie. Vele potentiële ovulatievoorspellers zijn bestudeerd maar alleen de bronstduur bleek een redelijke schatter voor het ovulatiemoment. Het nadeel van de bronstduur als ovulatievoorspeller is dat het een retrospectieve voorspeller is. Ovulatie vindt namelijk plaats op tweederde deel van de bronst.

De gemiddelde bronstduur varieerde van 31 tot 64 uur op verschillende bedrijven (Hoofdstuk 3.1). Bovendien bleek de bronstduur op een bedrijf consistent over maanden, met een herhaalbaarheid van 86%. Verder bleek dat de bronstduur op veel bedrijven negatief gerelateerd is met het interval spenen-bronst en dat deze relatie

verschillend is tussen bedrijven. Deze specifieke bedrijfsparameters kunnen worden gebruikt om de bronstduur van zeugen te voorspellen en daarmee het ovulatiemoment. Deze bedrijfsparameters (gemiddelde bronstduur en de relatie bronstduur en interval spenen-bronst) kunnen daarom worden gebruikt om een specifieke inseminatiestrategie voor ieder bedrijf te definiëren.

ONTWIKKELING VAN EEN MODEL VOOR INSEMINATIE STRATEGIEËN

Er zijn vele factoren die het reproductieproces beïnvloeden. Een beter inzicht in een dergelijk complex proces kan worden verkregen via simulatie-studies waarin de onderliggende processen gereguleerd kunnen worden. Een simulatie model voor inseminatie strategieën (PIG Simulation model for Insemination Strategies ; PIGSIS; Hoofdstuk 4.1) is ontwikkeld en bestaat uit twee delen: (1) de opeenvolgende gebeurtenissen in het reproductieproces vanaf het aantal geovuleerde eicellen tot aan het aantal biggen bij geboorte en (2) timing van inseminatie ten opzichte van ovulatie gebaseerd op de bedrijfsparameters (interval spenen-bronst, bronstduur, etc.). PIGSIS simuleert reproductieresultaten op dag 1, 5, 10, 15, 35 en 110 van de dracht. Veel fysiologische processen zoals bevruchting, embryonale sterfte (door degeneratie, maternale herkenning van de dracht en door embryonale baarmoedercapaciteit) en foetale sterfte (door foetale baarmoedercapaciteit) kunnen in PIGSIS worden gevarieerd om het effect op reproductieresultaten te bestuderen. Na verificatie en validatie kon worden geconcludeerd dat PIGSIS een robuust model is dat reproductieresultaten goed lijkt te simuleren. In de basis situatie (gemiddelde bronstduur van 47 uur en een gemiddeld worpnummer van 4.2) met een inseminatie tussen 0 en 24 uur voor ovulatie simuleert PIGSIS 12.9 totaal geboren biggen en een afbigpercentage van 94.9%. Onder deze omstandigheden is de gemiddelde embryonale en foetale sterfte van het aantal dieren dat drachtig was respectievelijk 34.9% en 3.0%. Het effect van het interval tussen inseminatie en ovulatie op de bevruchtingsresultaten is groot, maar dit effect wordt steeds minder groot naarmate de dracht langer is. Uiteindelijk is het effect van het inseminatiemoment op afbigpercentage groter dan op worpgrootte.

In de Algemene Discussie zijn de resultaten van de diverse studies in dit proefschrift bediscussieerd en een voorbeeld van de bruikbaarheid van PIGSIS is gegeven. De verificatie en de deel-validatie van PIGSIS gaven betrouwbare resultaten. Een verdere evaluatie van PIGSIS is noodzakelijk door middel van een gehele validatie. Daarom moet PIGSIS nog beschouwd worden als een model dat in de ontwikkelfase en voorzichtigheid moet worden betracht bij het gebruik van PIGSIS om een optimale inseminatie strategie voor bedrijven te definiëren.

Curriculum Vitae

Dorothé Wilhelmina Basilissa Steverink werd op 22 oktober 1967 geboren te Silvolde. In 1984 behaalde zij het MAVO diploma aan de Pastor Bleumers MAVO te Silvolde waarna in 1986 het HAVO en in 1988 het VWO diploma werd behaald aan het Isala College te Silvolde. In hetzelfde jaar begon zij met de studie Zoötechniek aan de Landbouwuniversiteit te Wageningen. In 1994 studeerde zij af, met als oriëntatie Veefokkerij en Gentechnologie. Als vervolg op haar afstudeervak veefokkerij is zij voor enkele maanden werkzaam geweest als toegevoegd onderzoeker in Foulum te Denemarken aan het National Institute of Animal Science. In mei 1995 werd zij aangesteld als Assistent In Opleiding (AIO) bij de vakgroep Veehouderij van de Landbouwuniversiteit en verrichtte zij het in dit proefschrift beschreven onderzoek. Sinds 1 juli 1999 is zij als geneticus werkzaam bij het Institute for Pig Genetics (IPG) te Beuningen.

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